

name	symbol	Applied Bioystems "assay on demand" assay #	forward primer	Seq ID No.	reverse primer	Seq ID No.	Seq ID No.	Seq ID No.
aspartin (lrr class 1)	ASPN		AATACAAAGGACACATCAAGGA	1	TCTTCTGCAATTTGATATGGA	23	TTGGAAATGAGTGCAAAAGCTCTGATATATG	45
chondroitin sulfate proteoglycan 2 (version)	CSPG2		GCCAGTGGATGATGTTCC	2	TCTTCTGCAATTTGATATGGA	24	AGGCAAGATTTGCTTGGGCGAC	46
cystatins SN, SA & S	CST1, 2, 4		AGTCCAGCCCACTTGGGA	3	GGGAAGCTTGTGATCTGGAAAGA	25	AGGCAAGATTTGCTTGGGCGAC	47
gamma-glutamyl hydrolase	GGH		GTGGCAATGGCGGTGA	4	TTGACGACCAACTCTAGTAGGAAA	26	TTGACGACCAACTCTAGTAGGAAA	48
heparin-like growth factor binding protein 7	IGFBP7		CAGGTCAAGCAAGGCGAC	5	TGACAGCTCAAGTACACCTGGG	27	AGGCAAGATTTGCTTGGGCGAC	49
heparin 10	KLK10		ACACATGATATGTCTGGAGTG	6	GAGAGGATGCTTGGAGGT	28	CTTCCAGAGTGAATCTTGGAGGCG	50
leucine proline-enriched proteoglycan 1 (leprecan 1)	LEPRE1		CTTGAGTACAGGCTGAGCTTC	7	CGGTGACACAGTCTCTTTACAG	29	CGATCAGAGTCACTTACATTCAGGCTGCA	51
lumican	LUM		GATCTCTGTGCATGTGCTATGC	8	CCATCAATGCGCAGGAGAGA	30	TAGGATTCAAAGCATTTGGCCAAAATGAGTGTAA	52
lyso oxidase-like 2	LOXL2		AGGCCAGCTCTGCTTGGGA	9	CCCTGATGCGCGAGTTG	31	CGTAACTCTGTGATGTCTCTTCAATCTG	53
matrix metalloproteinase 12	MMP12		GGCTCTGCTGTGATGATAGT	10	AGTGACAGCATCAAACTCAATTG	32	TCAGTCTCTGTATGTGAGAGCCCAAAAGAGAA	54
metalloproteinase inhibitor 1	THP1		CGAGACCACTTATACACGG	11	GGAGCTGTGGAGTATGCGC	33	CAAGATGACCAAGATGATATAAGGGTTTCAAGC	55
n-acylsphingosine amidohydrolase	ASAH1		GGCAGACGCTTGCAAA	12	ACAGGACATCATATGTTTCAAA	34	TGTTGAGACGACACAGCCAGAGAGAATA	56
secreted frizzled-related protein 2	SFRP2		GGTACGACGAGCAAGCT	13	TTTGGAGGCTTCAATAGCTTT	35	CTGGCAGGCGAAGGAGGAGGCTC	57
secreted protein, acidic, cysteine rich	SPARC		TTCTCCGTGACAGTGGCATTC	14	GAAAAGCGGGTGGTGA	36	TTGGACGAGCGAGCCATTTGAGG	58
serpine protease 11 (IGF binding)	PRSS11		TGGGAGCGCGGTTAGTAA	15	AAGGAGTTCCAGCTGTCTCTTC	37	AGTGTAAITTCATCACTTCCAGGCTGAGG	59
thrombospondin 2	THBS2		TGGAGGAGTACAGGCGCTATAG	16	TAGGTTTGCTCATAGTAGGTTCTGAGT	38	AGGCGCAGAGCGGCTACATCAGATC	60
thyroglobulin	TG		GAGGTTCTCTGCGAGTTCAA	17	TGTAAAGCGCTCCACTTCACAT	39	TGTGGCAGATTTCAGATGCGCCAAA	61
human cell growth regulator with EE hand domain 1	GR11		CTGCGCACCCCTTCCA	18	TTCTGTCTCTCTAGTCTTTTGGG	40	CGAGGCGAGGAGGAGGCTGG	62
human serine or cysteine proteinase inhibitor clade B	SERPINF5		TCGACGATTTTCCAGGATAA	19	AAGCGCAATTTGCTAGTGTGA	41	TCAGTCCAGGCGCGCGCAATGGA	63
transforming growth factor beta 1	TGFB1		GGTCCATGTCATCCACATGTT	20	TCTGCAAGTTTCTCCCTCTTT	42	CAAGCTCCAGCCACAGAGCTCAGG	64
human proprotein convertase subtilisin/kexin type 5	PCSK5		AAAATCTTGCCCGGAAATGC	21	JAGTCTGGCGGTTGGAATAGC	43	ACAGAAATGAGGATGGGTTAGGCTGCA	65
matrix metalloproteinase 2	MMP2		TTGATGCGATGCTCAGATC	22	TGTCAAGTGGCGTCAAGT	44	TTCAAGGACCGGTTCACTTTTGGGG	66
human serine or cysteine proteinase inhibitor clade H	SERPINF1	Hs00241844 ml						
adipon		Hs00377849 ml						
egf-containing fibulin-like extracellular matrix protein 2	EFEMP2	Hs00213545 ml						
secreted frizzled-related protein 4	SFRP4	Hs00180066 ml						
inhibin beta A chain	INHBA	Hs00170103 ml						
osteopontin	OPN	Hs00167093 ml						
transforming growth factor beta-induced	TGFB1	Hs00165908 ml						

Figure 1

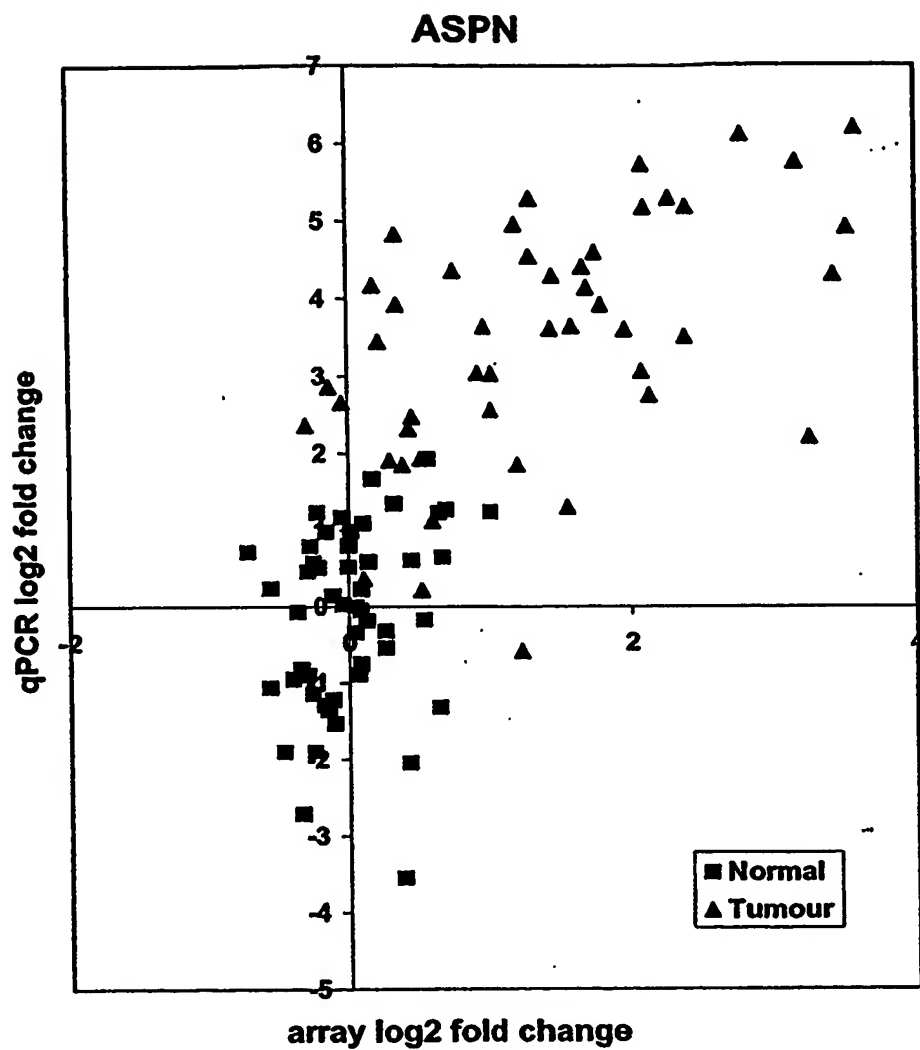
Abstract - Identification of Markers for Gastric Malignancy

Microarray - Identification of Markers for Gastric Malignancy										NCBI	protein ref	fold change	fold change rank	original t-test	Bonferroni-adjusted p value	2 sample Wilcoxon test
name		symbol	MWG oligo #	mRNA ref sequence	protein ref sequence	fold change	fold change rank	original t-test	Bonferroni-adjusted p value	2 sample Wilcoxon test						
adiponectin		ADPN	C:0531	NM_015419	NP_060150	1.8	-17818	1.0E-28	3.04E-24	0.0E+00						
angiotensin (rr class 1)		ANG	A:07749	NM_017680	NP_060150	2.6	-22292	6.4E-23	1.9E-18	0.0E+00						
carboxypeptidase N		CPN2	B:4922	-	P22792	2.7	-22367.5	2.3E-42	7.0E-38	0.0E+00						
cell growth regulatory factor with EF-hand domain		CGRL1	A:07876	NM_006569	NP_006560	3.0	-21188.5	4.33E-42	1.3E-37	0.0E+00						
chondroitin sulfate proteoglycan 2 (versican)		CSG2	A:10008	NM_004385	NP_004376	2.3	-21606.5	2.23E-33	6.65E-29	0.00E+00						
cystatin SN		CST1	A:06089	NM_001889	NP_001889	2.1	-17475	1.3E-18	3.8E-14	0.0E+00						
cystatin SA		CST2	A:06089	NM_001322	NP_001313	2.1	-17475	1.3E-18	3.8E-14	0.0E+00						
cystatin S		CST4	A:06089	NM_001899	NP_001899	2.1	-17475	1.3E-18	3.8E-14	0.0E+00						
efg-containing fibulin-like extracellular matrix protein 2		EFEMP2	A:09072	NM_016938	NP_058634	2.4	-22761	2.0E-35	5.9E-31	0.0E+00						
gamma-glutamyl hydrolase		GGH	A:03601	NM_003878	NP_003869	1.6	-18092	1.6E-07	4.8E-03	5.7E-11						
inhibin beta A chain		INHBA	A:02189	NM_002192	NP_002183	2.1	-21247	1.4E-30	4.3E-26	0.0E+00						
insulin-like growth factor binding protein 7		IGFBP7	A:03385	NM_001553	NP_001544	3.0	-25854	5.4E-31	1.6E-26	0.0E+00						
kalikrein 10		KLK10	A:07907	NM_002776	NP_002767	2.3	-17986.5	5.0E-10	1.5E-05	4.9E-06						
leucine proline-enriched proteoglycan 1 (leprecan 1)		LEPRE1	A:04646	NM_022356	NP_071751	1.7	-18019	8.2E-14	2.4E-09	1.1E-12						
lumican		LUM	A:09199	NM_002345	NP_002336	2.9	-24927	4.2E-24	1.3E-19	0.0E+00						
lysyl oxidase-like 2		LOXL2	A:06085	NM_002318	NP_002309	1.6	-16994.5	5.9E-10	1.7E-05	7.9E-10						
matrix metalloproteinase 2		MMP2	A:06749	NM_004530	NP_008253	1.8	-18710	1.2E-11	3.6E-07	1.5E-10						
matrix metalloproteinase 12		MMP12	A:01762	NM_002426	NP_002417	2.1	-20209.5	2.2E-12	6.6E-08	4.9E-11						
metalloproteinase inhibitor 1		TIMP1	A:08048	NM_003254	NP_003245	3.2	-24177	7.5E-38	2.3E-33	0.0E+00						
n-acylsphingosine amidohydrolase		ASAH1	A:10030	NM_004315	NP_004306	1.7	-19636.5	9.6E-16	2.9E-11	0.0E+00						
olfactomedin		OLFM1	B:3555	NM_014279	NP_055094	3.9	-25782.5	6.5E-46	1.9E-41	0.0E+00						
osteopontin		SPP1	A:09441	NM_000582	NP_000573	7.0	-26668	4.0E-32	1.2E-27	0.0E+00						
human proprotein convertase subtilisin/kexin type 5		PCSK5	A:07004	NM_006200	NP_006204	1.7	-18736	2.0E-11	6.0E-07	7.3E-11						
group xiii secreted phospholipase a2		PLA2G12b	B:1811	NM_032562	NP_115951	3.0	-23212	7.92E-39	2.36E-34	0.00E+00						
secreted frizzled-related protein 2		SFRP2	B:1634	XM_050625	XP_050625	2.1	-19217	2.7E-10	8.1E-06	4.1E-08						
secreted frizzled-related protein 4		SFRP4	A:07398	NM_003014	NP_003005	3.0	-22153	6.0E-24	1.8E-19	0.0E+00						
serine (or cysteine) proteinase inhibitor clade H		SERPINH1	A:06615	NM_001235	NP_001226	1.9	-20252	2.8E-34	8.2E-30	0.0E+00						
human serine or cysteine proteinase inhibitor clade 8		SERPINH5	A:10485	NM_002639	NP_002639	1.5	-17026	4.6E-06	1.4E-01	5.6E-06						
serine protease 11 (IGF binding)		PRSS11	B:1274	NM_002775	NP_002766	1.6	-17184.5	9.3E-18	2.8E-13	0.0E+00						
secreted protein, acidic, cysteine rich		SPARC	A:08092	NM_003118	NP_003109	2.5	-22947.5	1.5E-44	4.6E-40	0.0E+00						
spondin 2		SPON2	B:2543	NM_012445	NP_036577	2.4	-20390.5	2.9E-31	8.5E-27	0.0E+00						
stannin		SNN	A:09316	NM_003498	NP_003489	2.1	-20162.5	3.25E-24	9.71E-20	0.00E+00						
thrombospondin 2		THBS2	B:9017	NM_003247	NP_003238	2.6	-22095	5.8E-29	1.7E-24	0.0E+00						
thrombospondin repeat containing 1		TSRC1	B:7686	NM_019032	NP_061905	2.6	-22608	1.38E-45	4.1E-41	0.0E+00						
thyroglobulin		TG	B:5402	NM_003235	NP_003226	2.4	-23644	4.39E-36	1.3E-31	0.0E+00						
transforming growth factor beta-induced		TGFB1	A:08124	NM_000358	NP_000349	2.5	-23339.5	1.96E-24	9.71E-20	0.0E+00						
transforming growth factor beta1		TGFB1	A:07050	NM_000660	NP_011137	1.6	-17214	2.30E-18	6.86E-14	0.0E+00						
hyaluronan and proteoglycan link protein 4		HAPLN4	C:6300	NM_023002	NP_075378	3.4	-23516.5	7.32E-44	2.2E-39	0.0E+00						
										Figure 2						

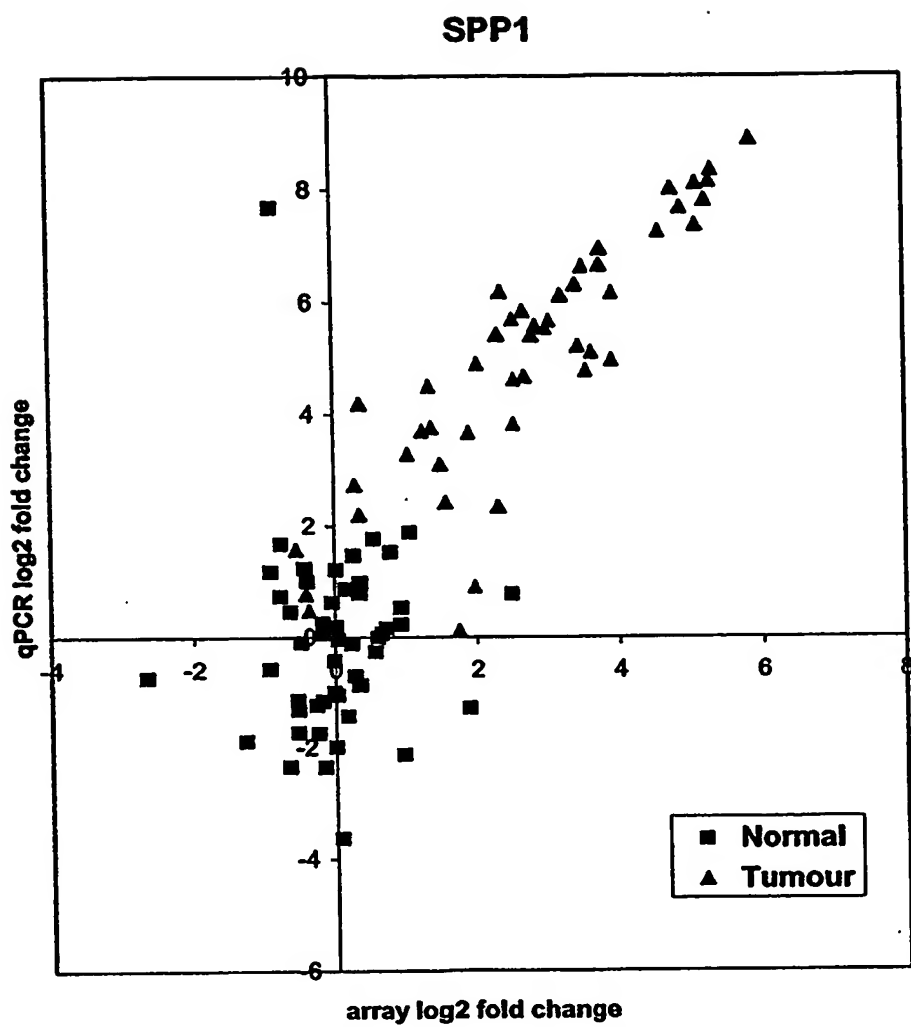
Figure 2

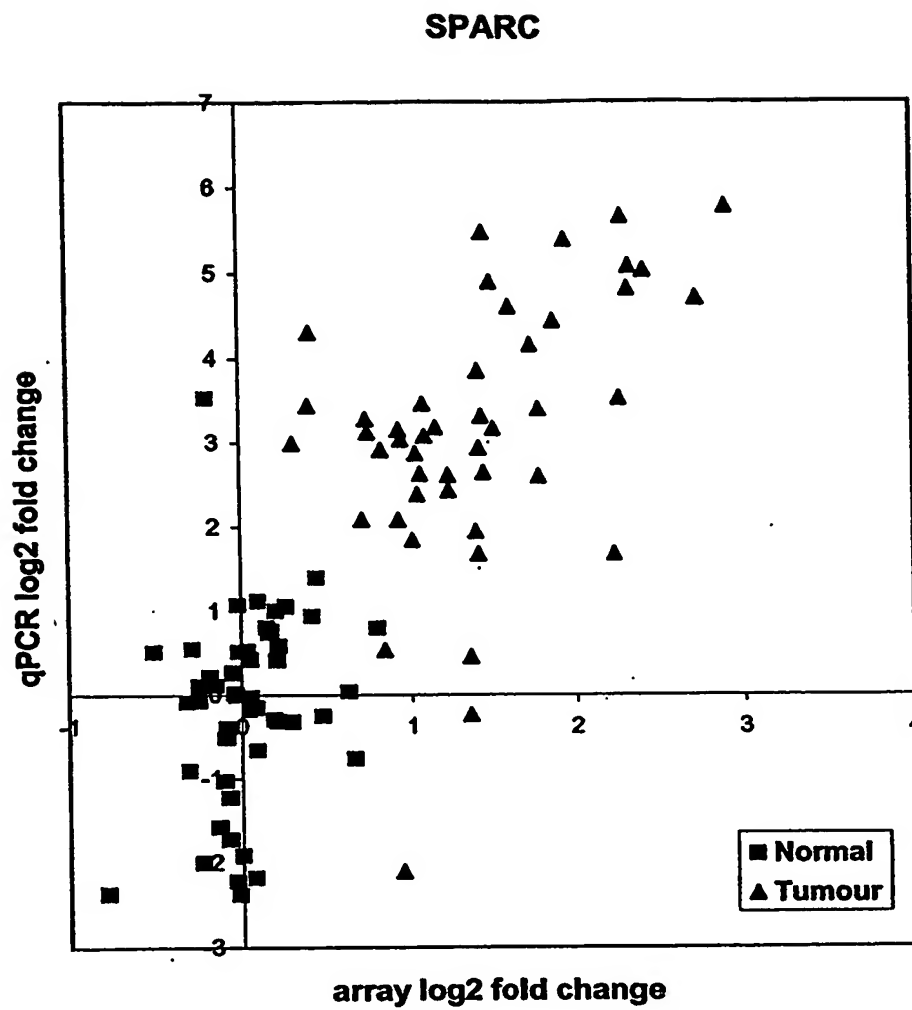
Quantitative RT-PCR - Quantification of Expression of Selected Gastric Cancer Candidate Genes				
name	symbol	median T:N fold change	Maximum T:N fold change	% T >95th percentile
adilcan		5	37	74
asporin (lrr class 1)	ASPN	12	73	91
chondroitin sulfate proteoglycan 2 (versican)	CSPG2	6	24	78
cystatins SN, SA & S	CST1, 2, 4	525	25532	100
egf-containing fibulin-like extracellular matrix protein 2	EFEMP2	3	15	56
gamma-glutamyl hydrolase	GGH	5	36	67
inhibin beta A chain	INHBA	34	357	98
insulin-like growth factor binding protein 7	IGFBP7	4	19	80
kalikrein 10	KLK10	5	633	70
leucine proline-enriched proteoglycan 1 (leprecan 1)	LEPRE1	4	17	72
lumican	LUM	5	47	80
lysyl oxidase-like 2	LOXL2	6	26	93
matrix metalloproteinase 12	MMP12	9	586	67
metalloproteinase inhibitor 1	TIMP1	8	19	91
n-acylsphingosine amidohydrolase	ASAH1	3	7	63
osteopontin	SPP1	40	481	96
secreted frizzled-related protein 2	SFRP2	5	85	63
secreted frizzled-related protein 4	SFRP4	56	600	100
secreted protein, acidic, cysteine rich	SPARC	9	56	93
serine protease 11 (IGF binding)	PRSS11	4	25	54
thrombospondin 2	THBS2	25	239	91
thryoglobulin	TG	5	153	54
transforming growth factor B-induced	TGFBI	7	204	82
1 percentage of tumors with expression levels greater than the 95th percentile of non-malignant samples.				

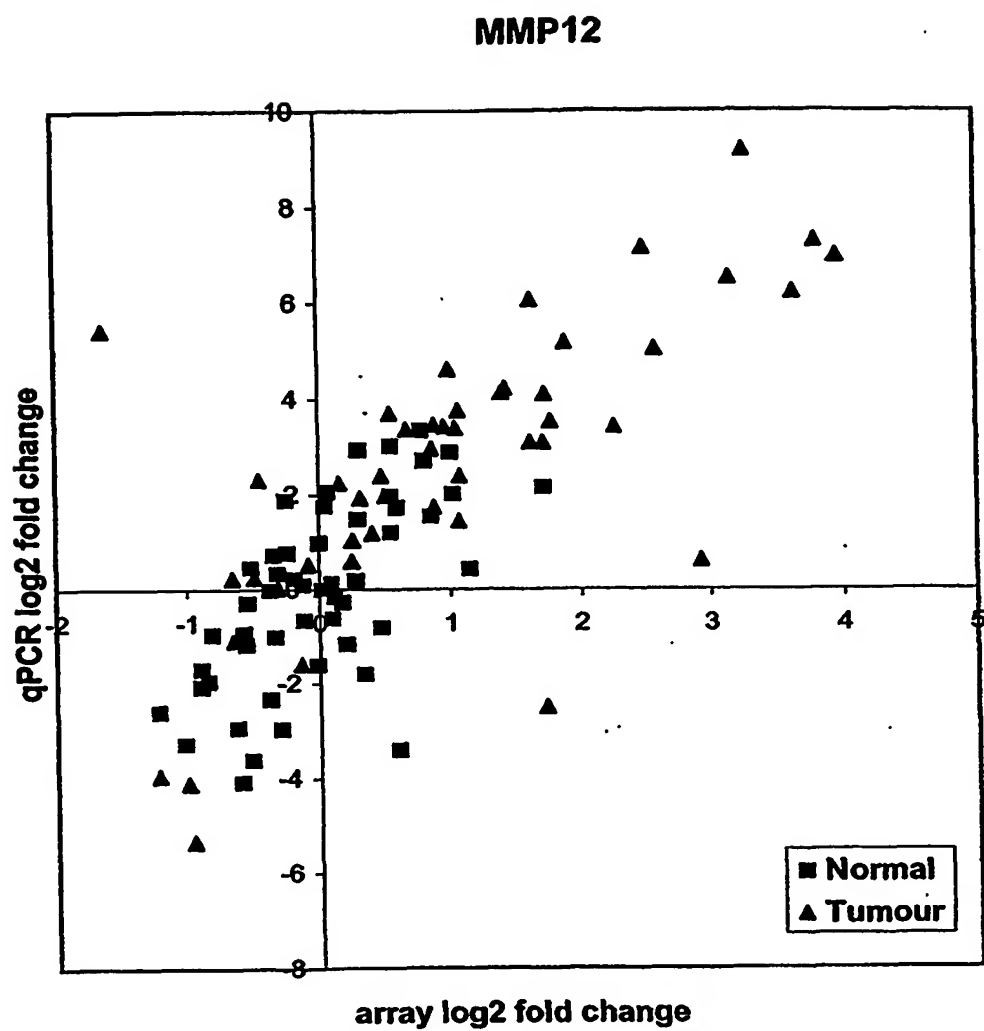
Figure 3

**Figure 4(a)**

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**Figure 4(b)**

**Figure 4(c)**

**Figure 4(d)**

ASPEN-tumor:median normal log2 fold change

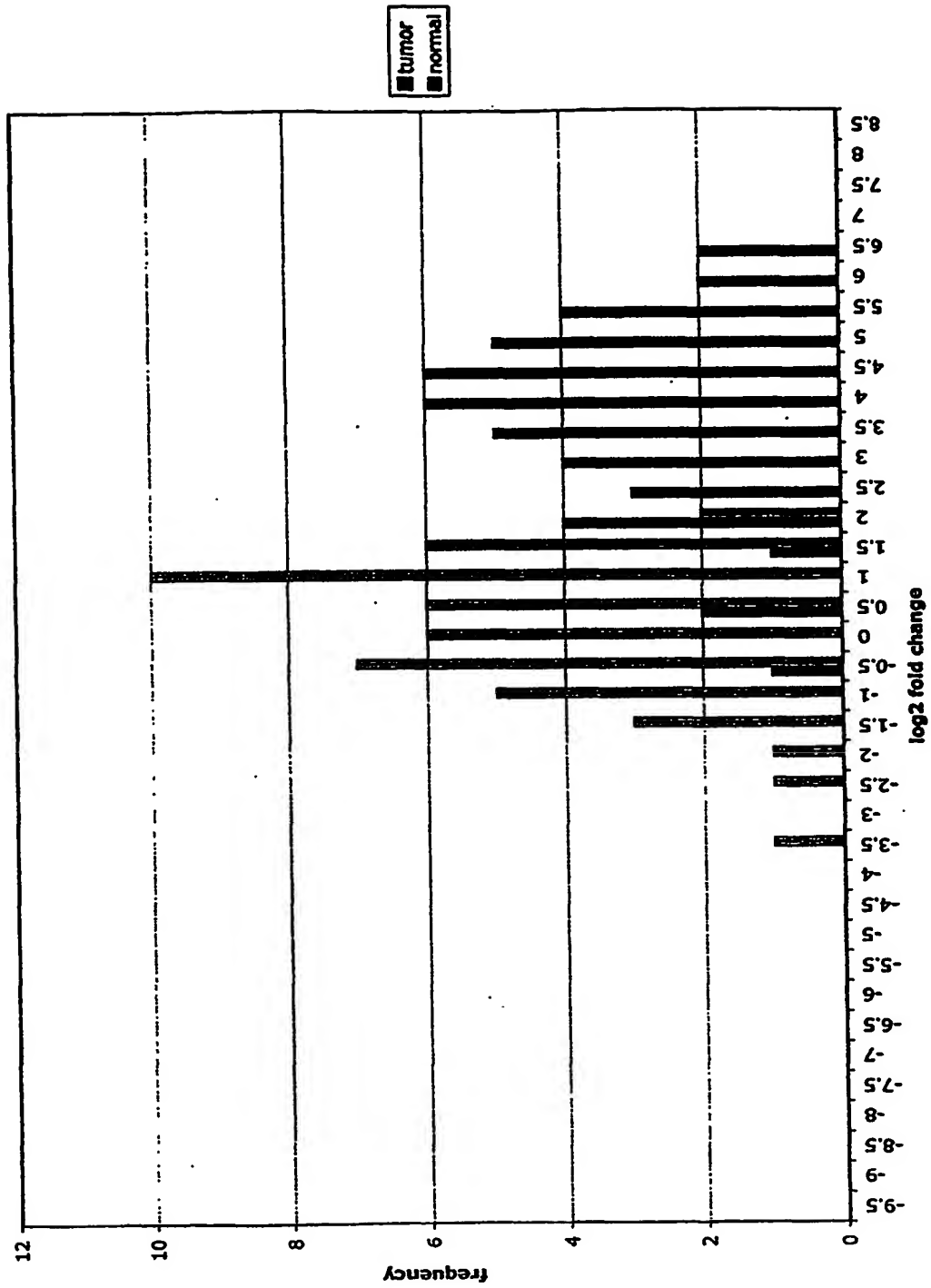


Figure 5(a)

CST1,2 & 4-tumor:median normal log2 fold change

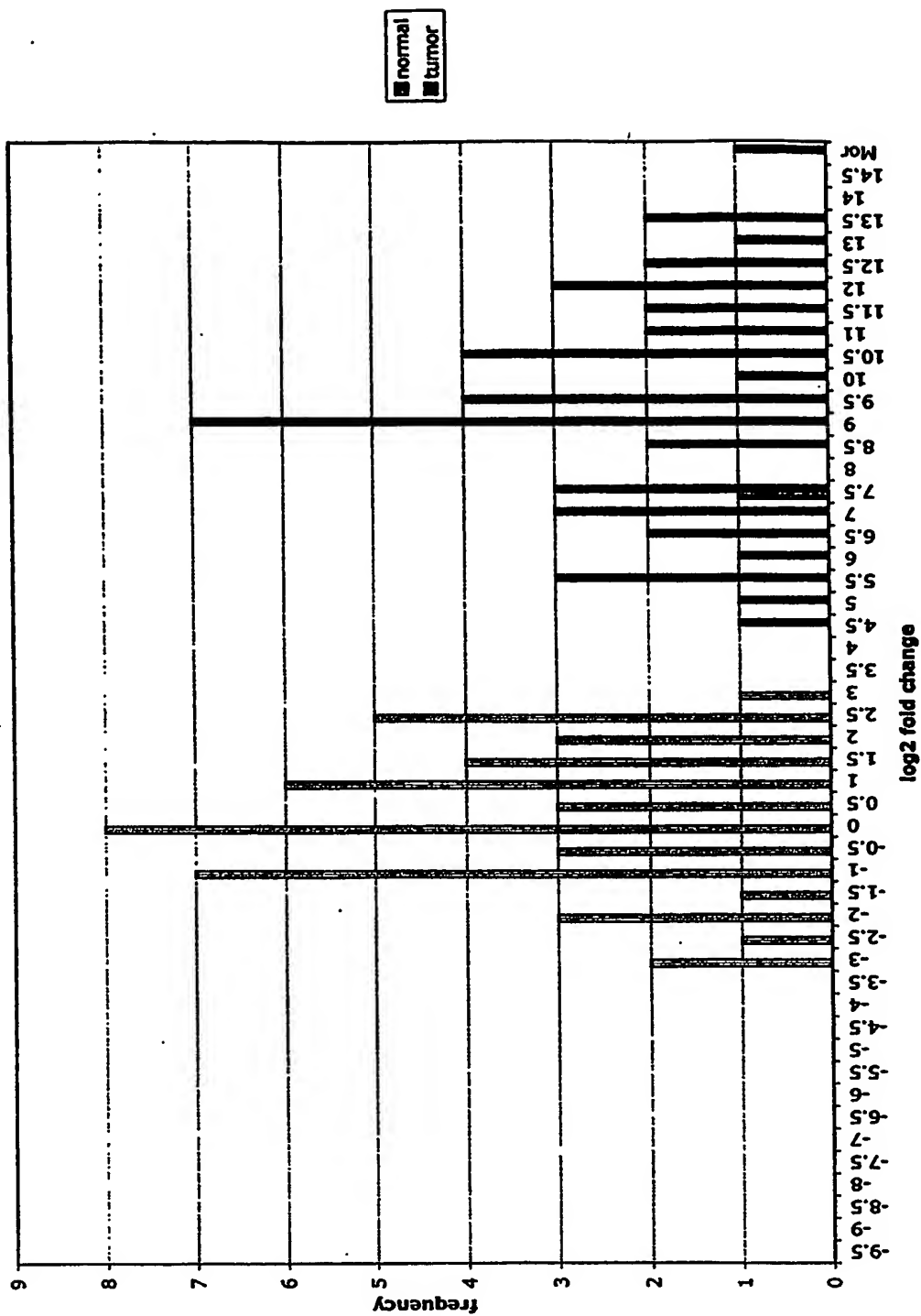


Figure 5(b)

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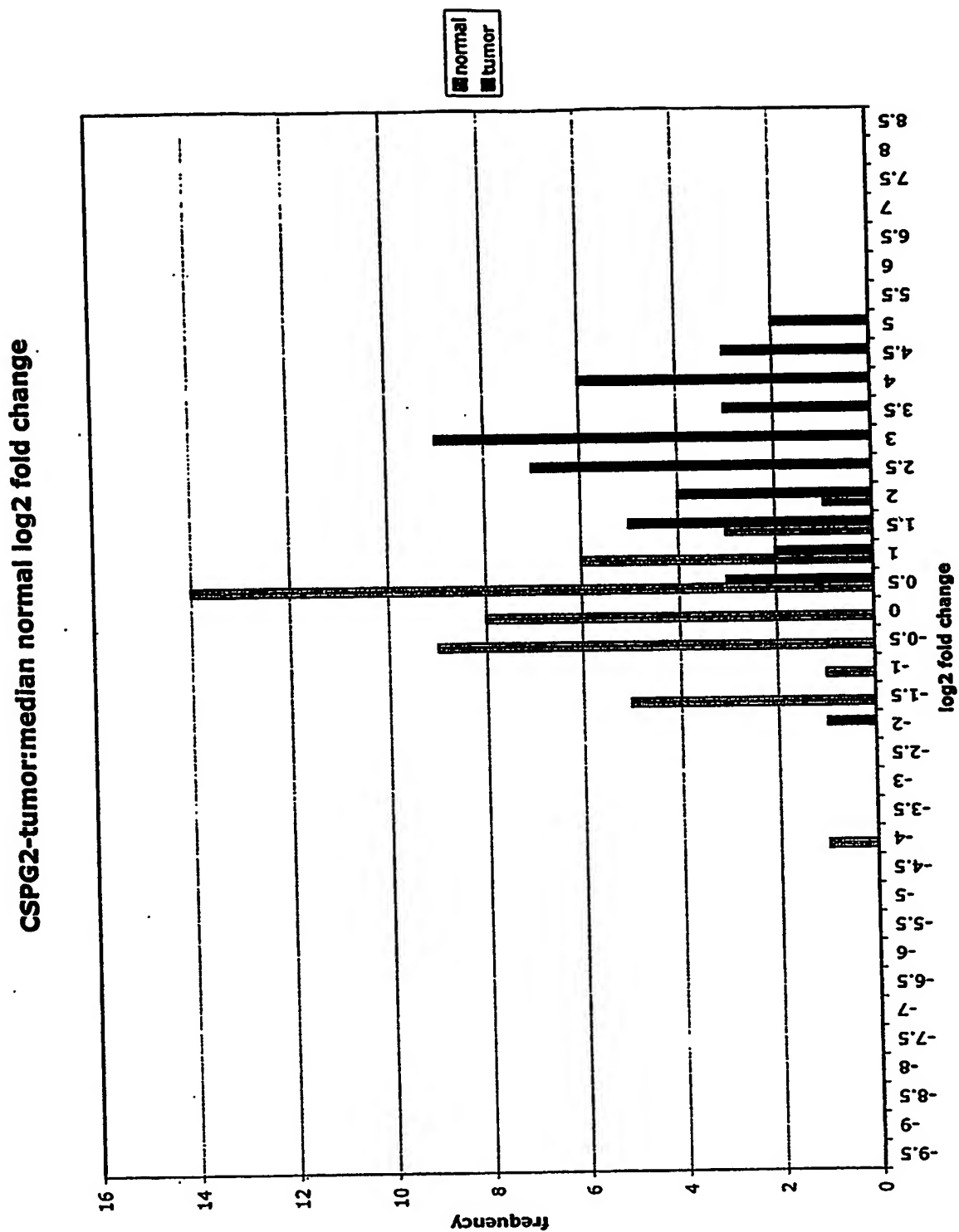


Figure 5(c)

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IGFBP7-tumor:median normal log2 fold change

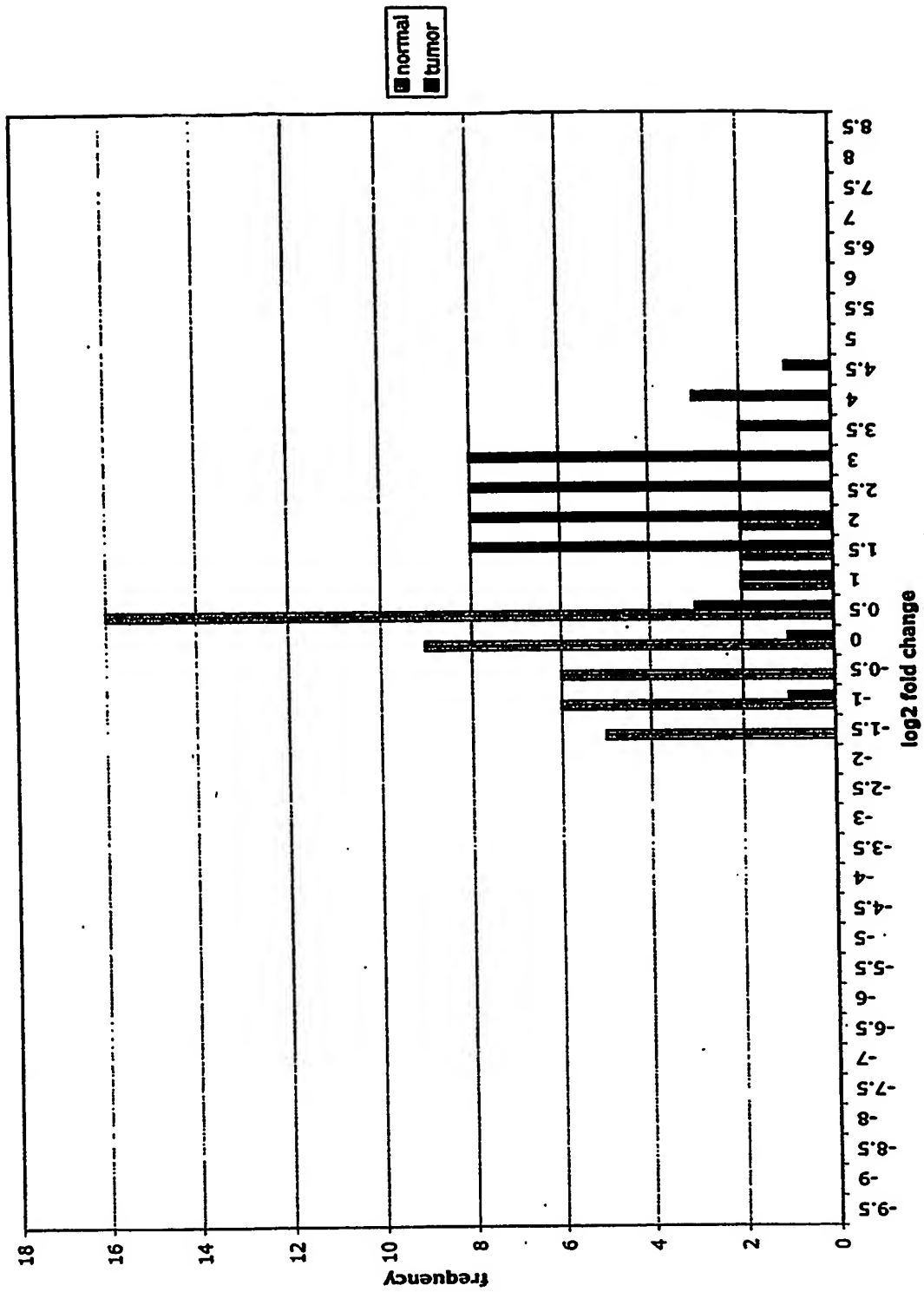


Figure 5(d)

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INHBA-tumor:median normal log2 fold change

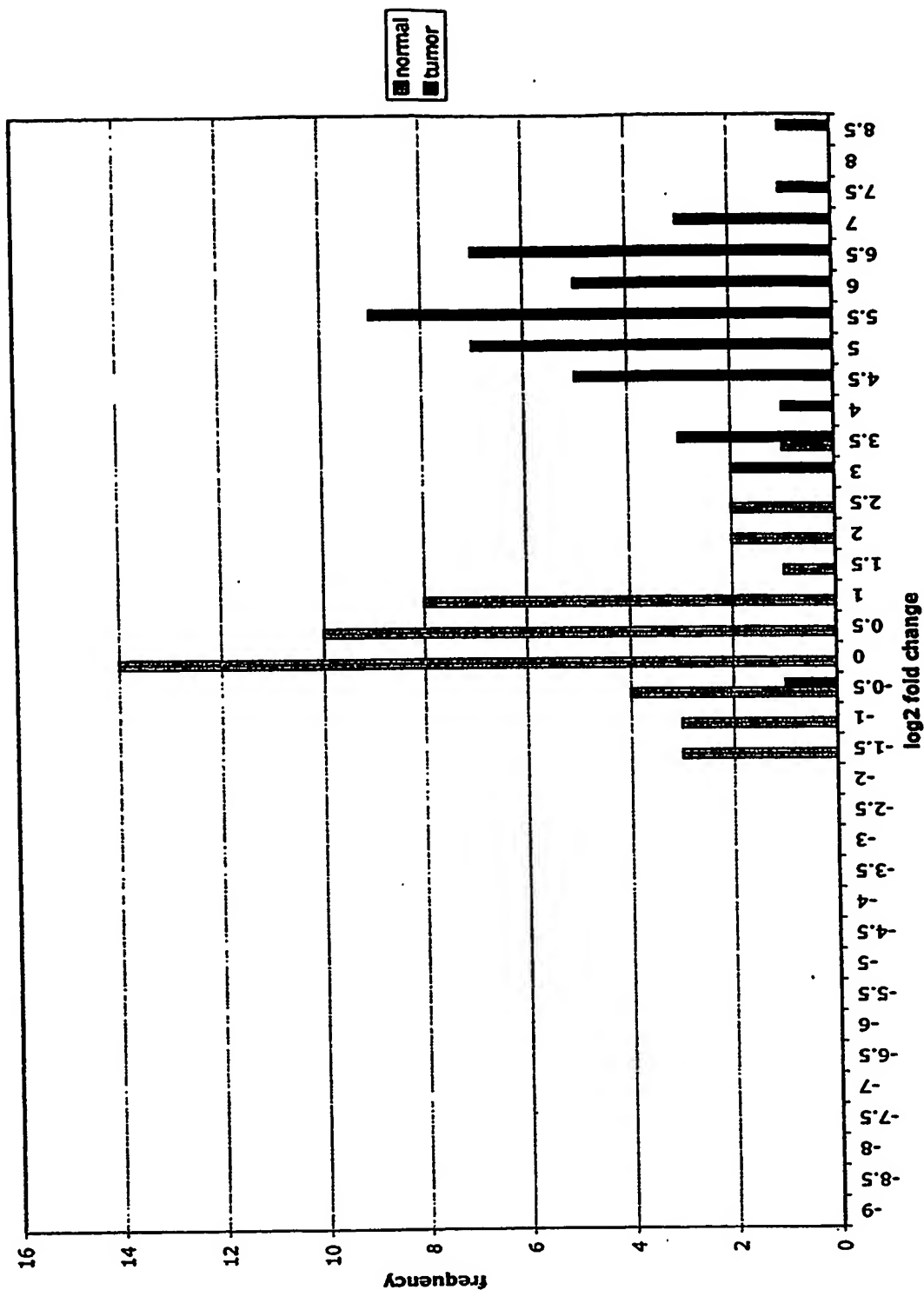


Figure 5(e)

LOXL2-tumor:median normal log2 fold changes

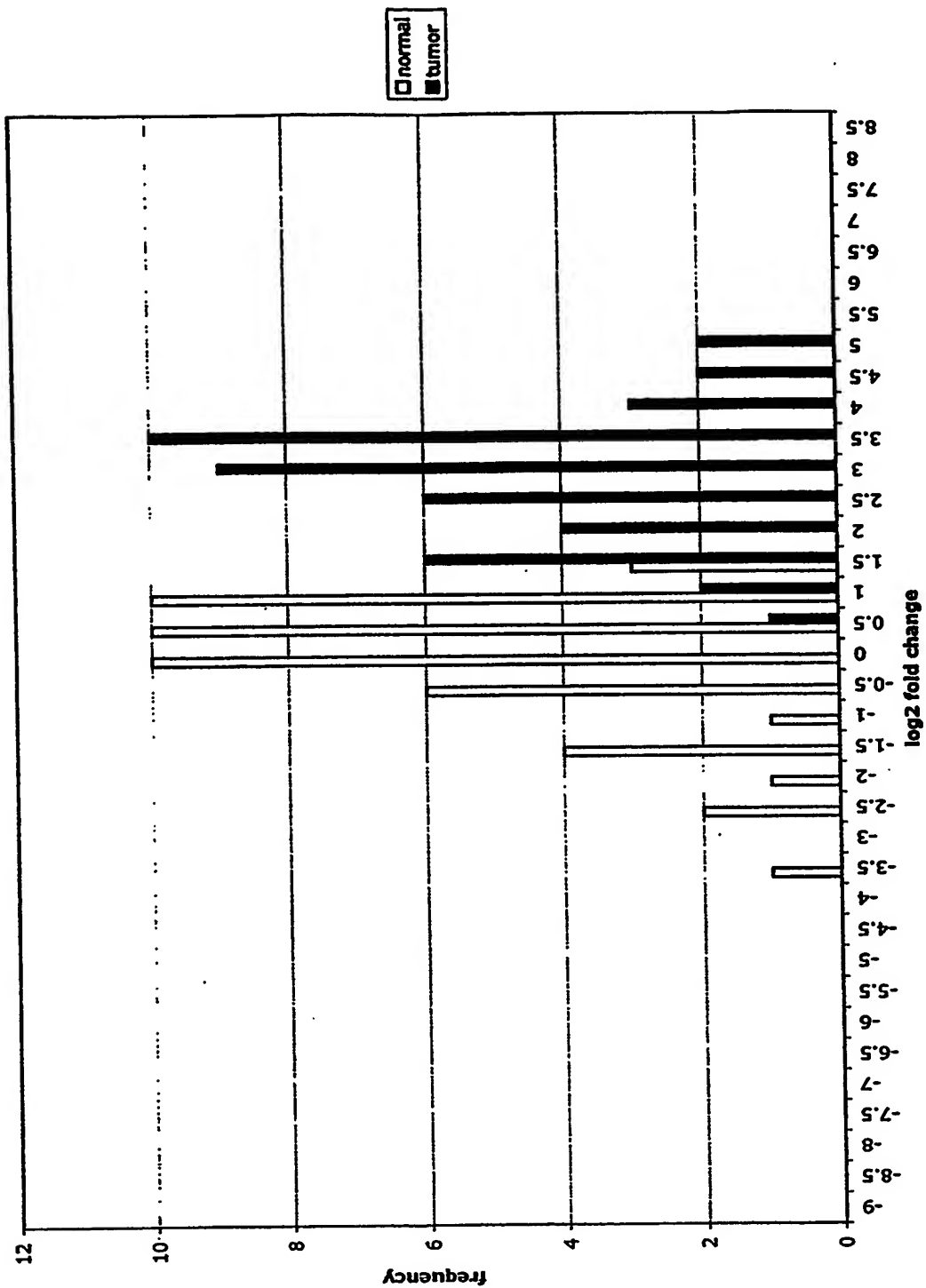


Figure 5(f)

lumican-Tumor:median normal log2 fold changes

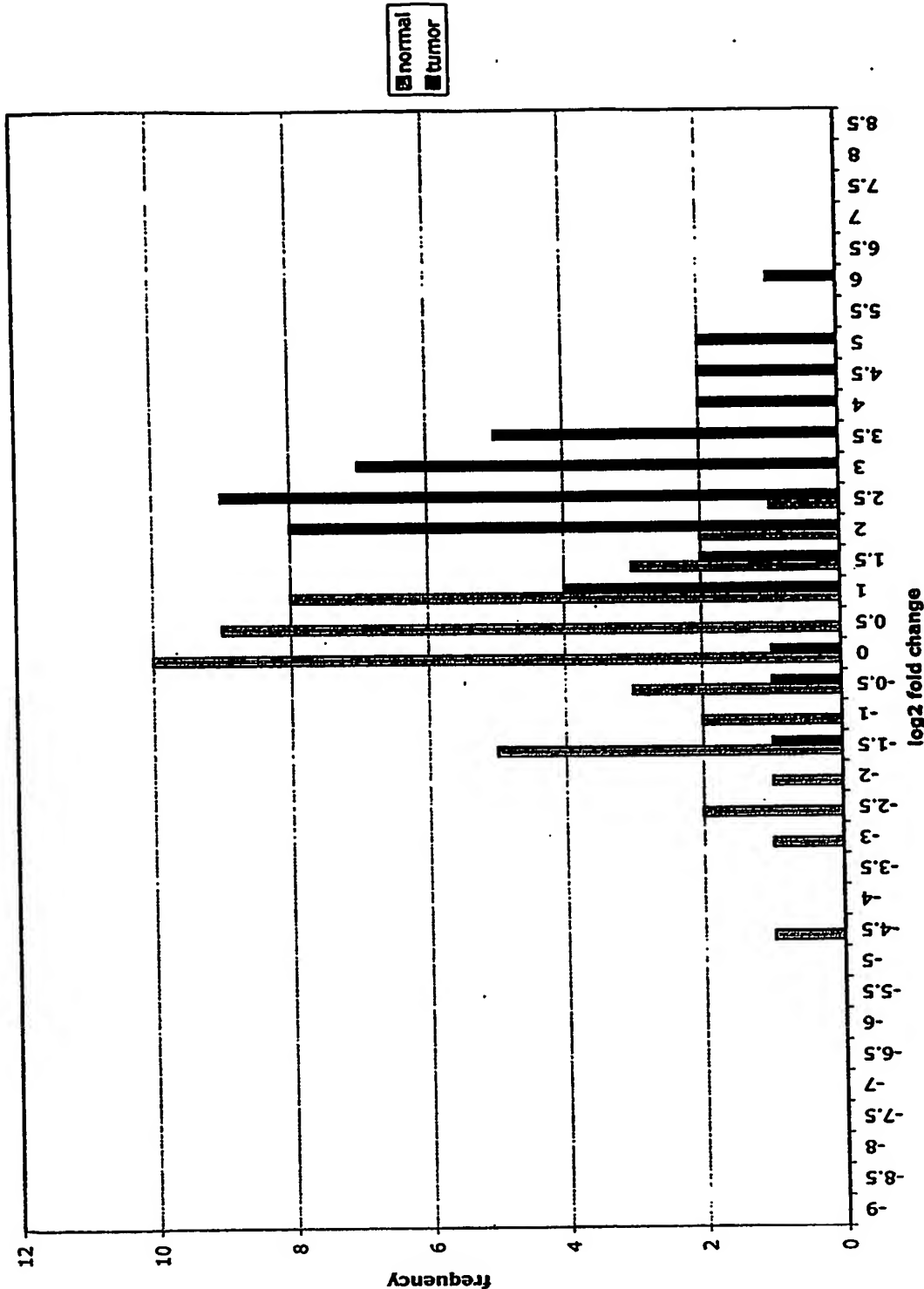


Figure 5(g)

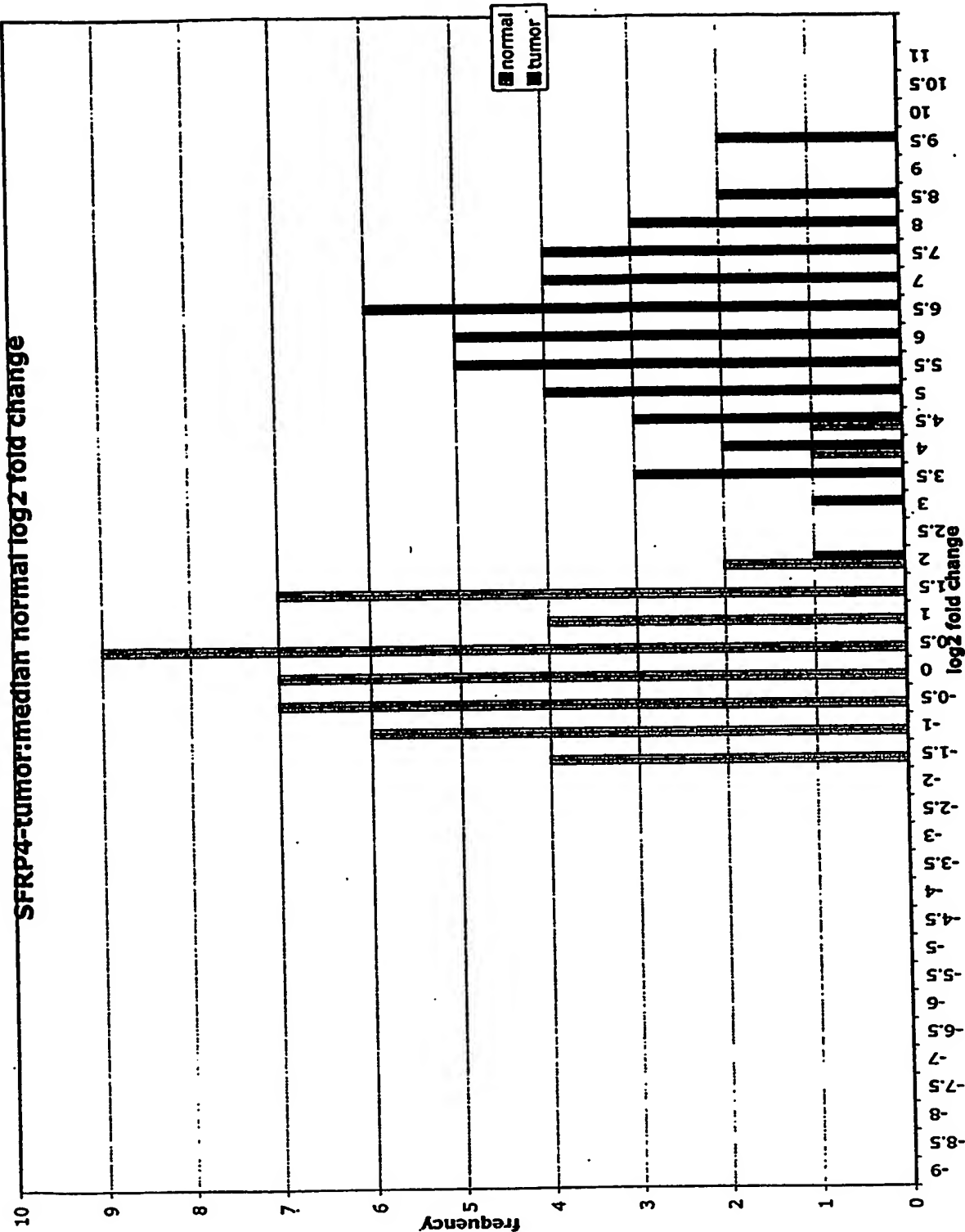


Figure 5(h)

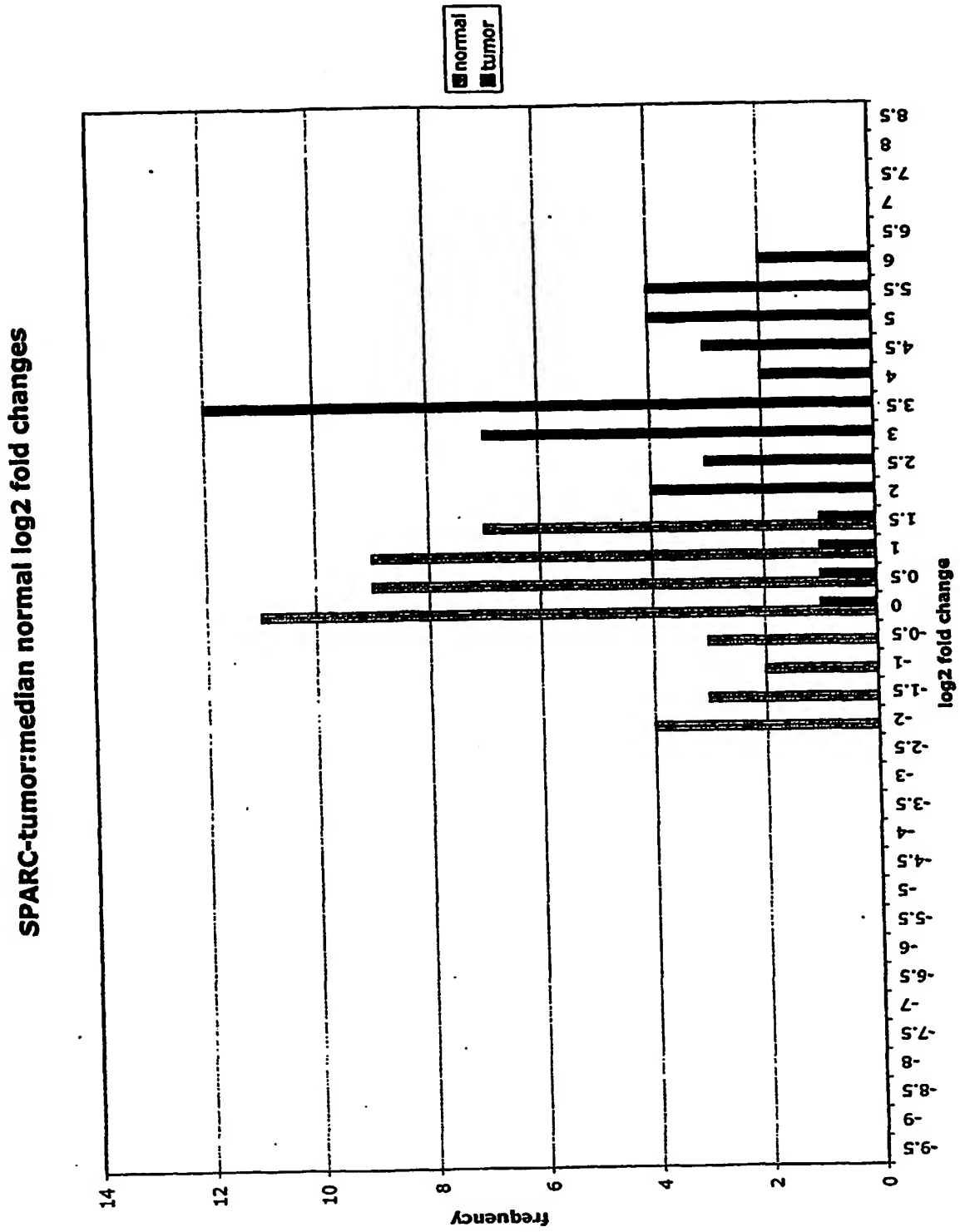


Figure 5(i)

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SPP1-tumor:median normal log2 fold change

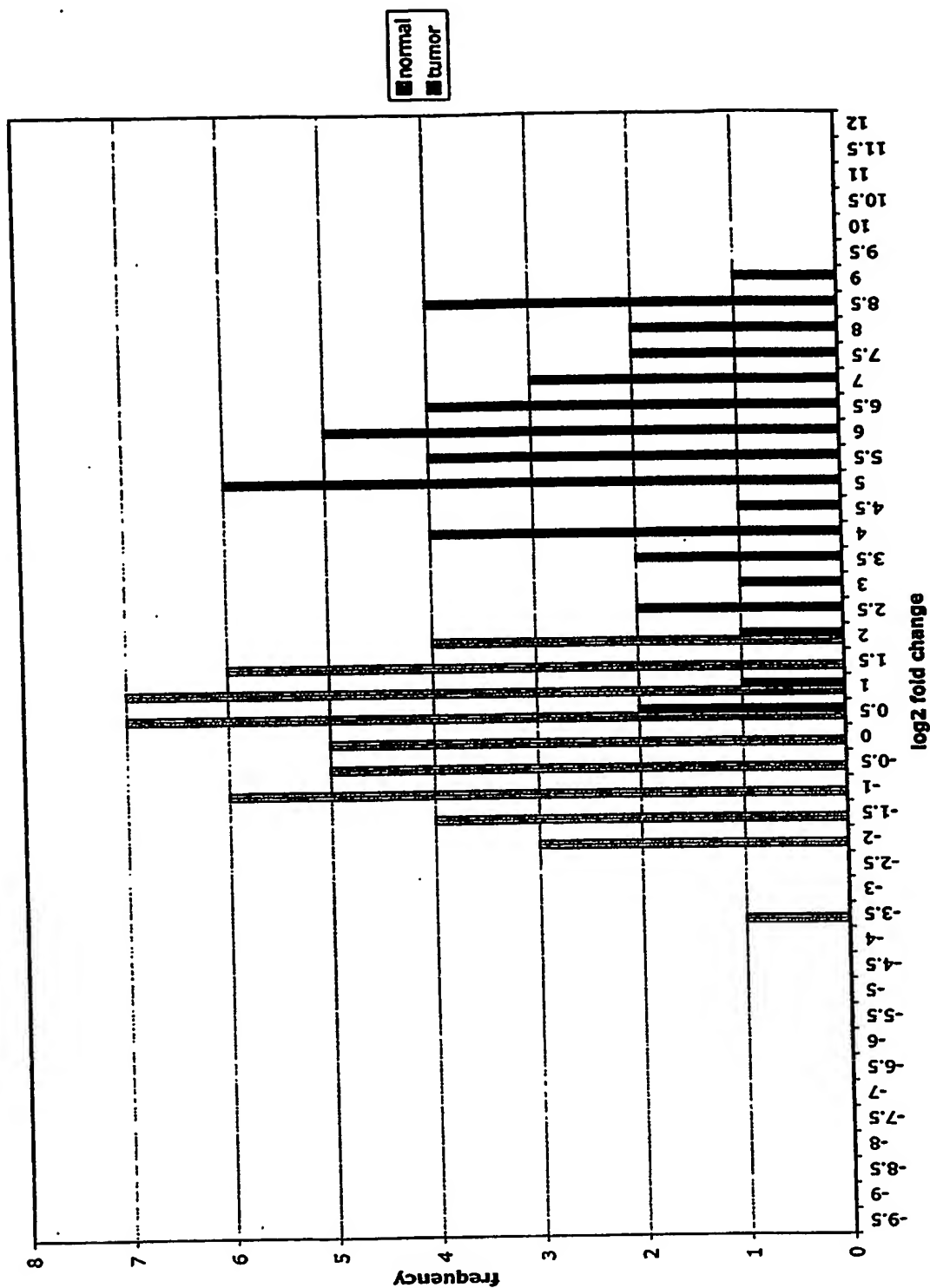


Figure 5(i)

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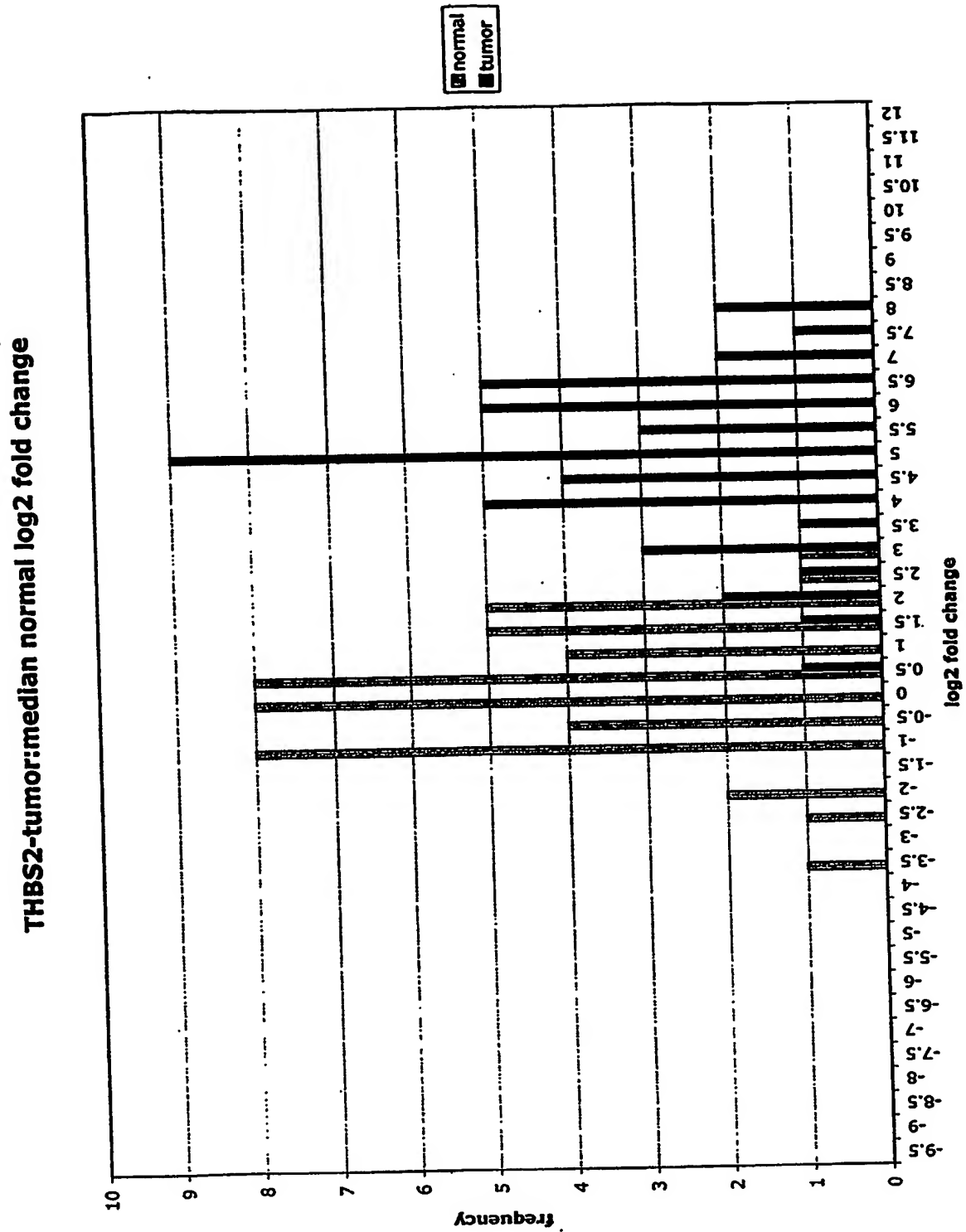


Figure 5(k)

TIMP1-tumor:median normal log2 fold change

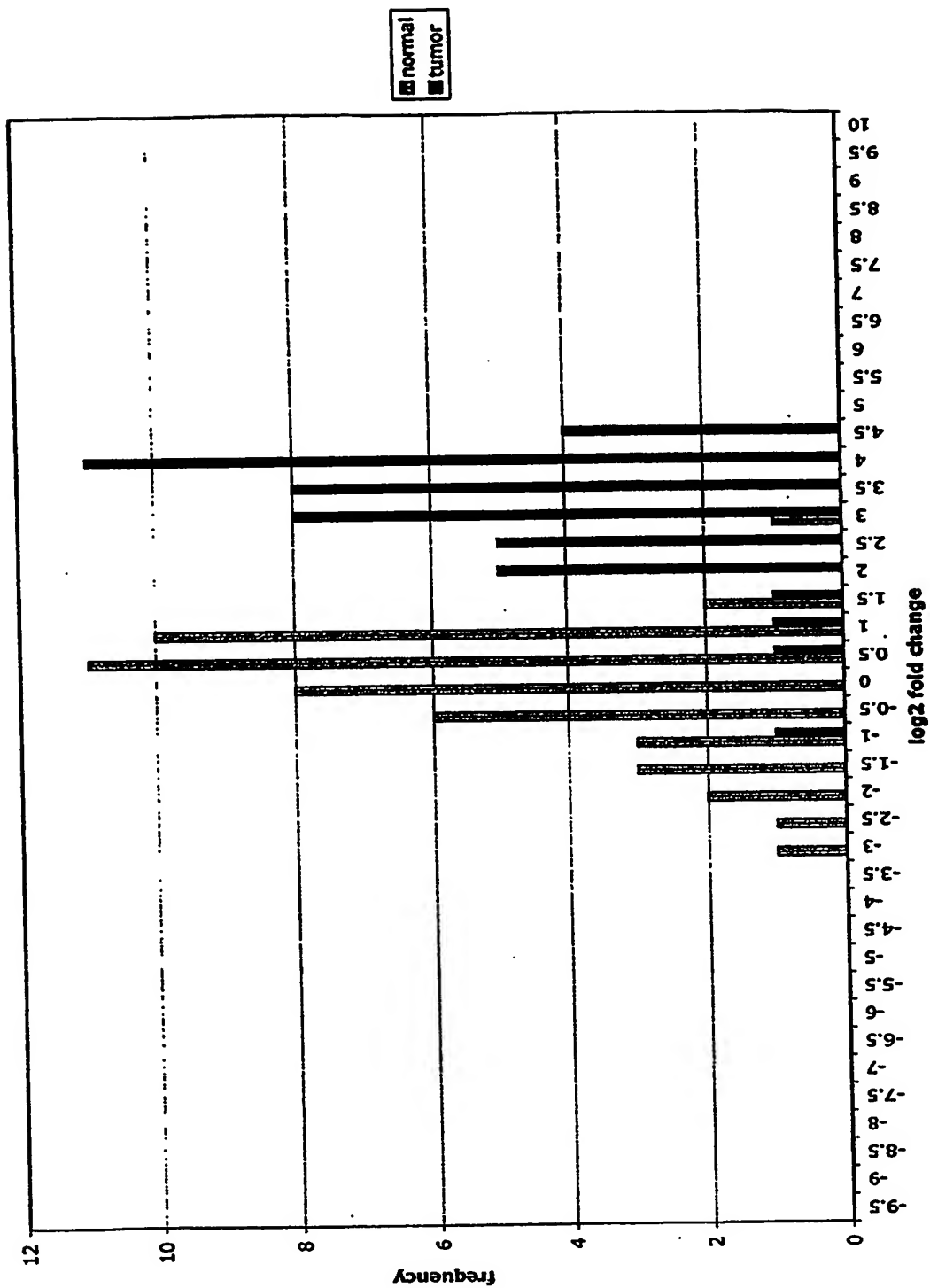


Figure 5(1)

adlcan-tumor:median normal log2 fold change

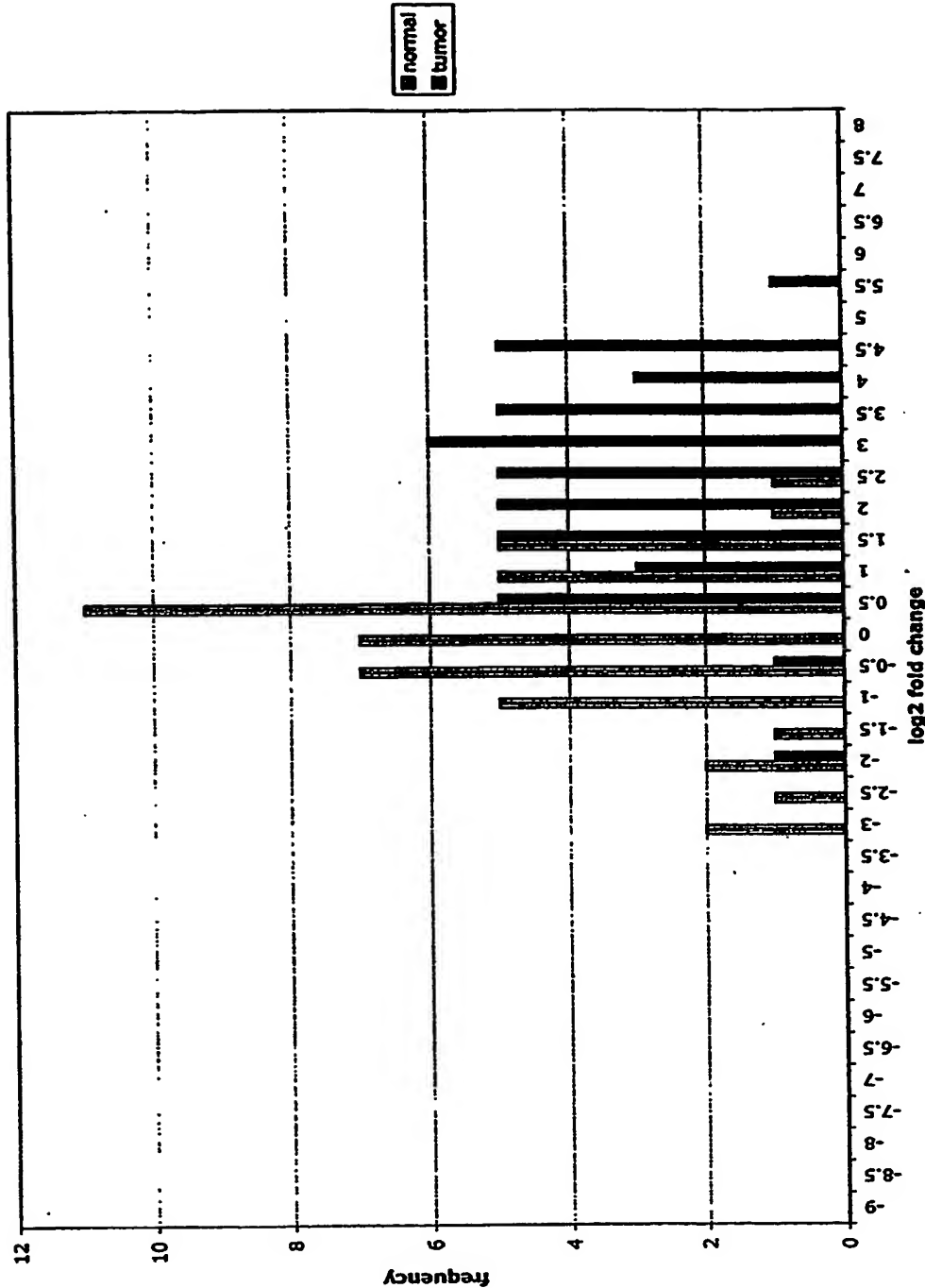


Figure 5(m)

PRS11- tumor:median normal log2 fold change

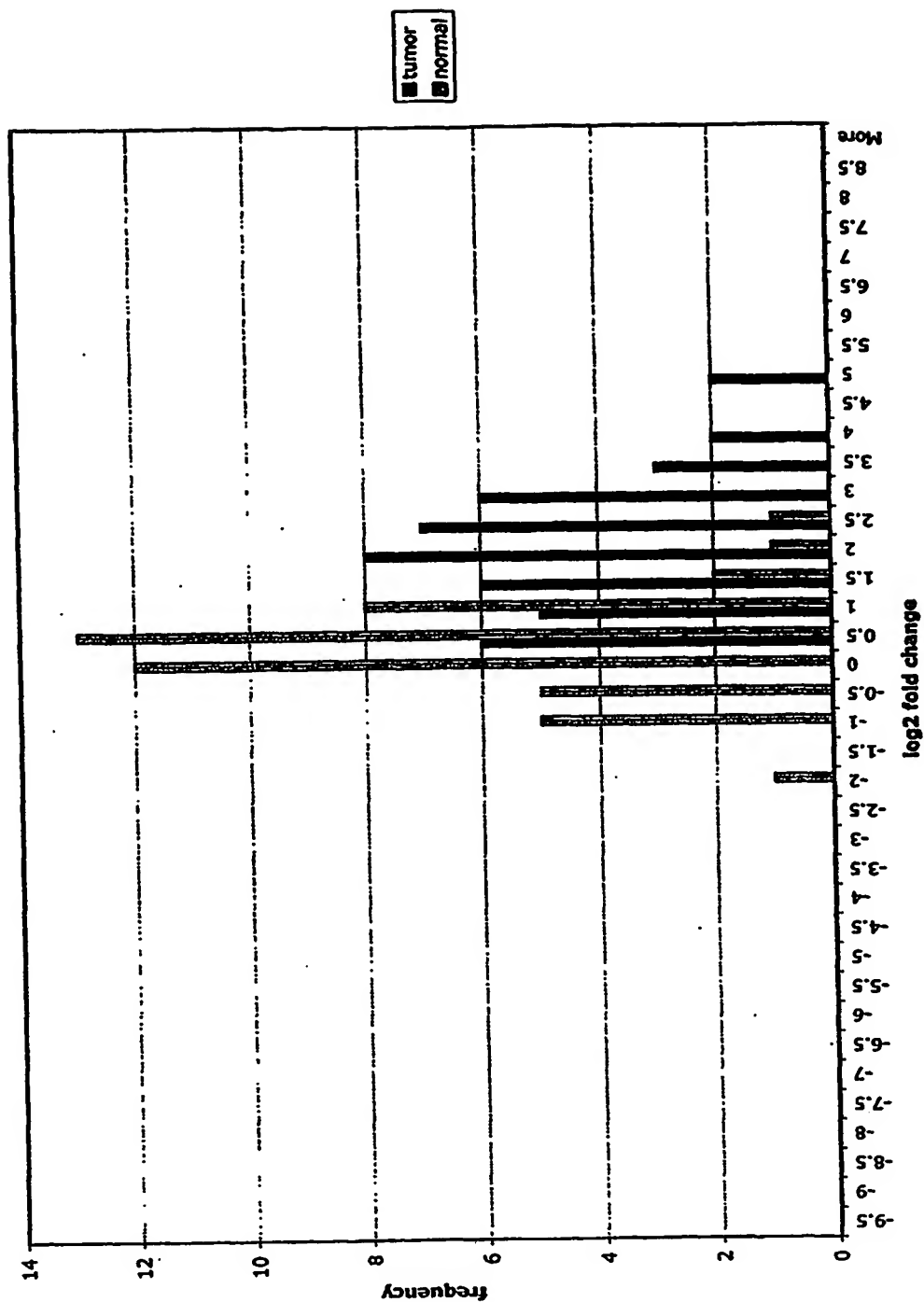


Figure 5(n)

ASAH1-tumor:median normal log2 fold changes

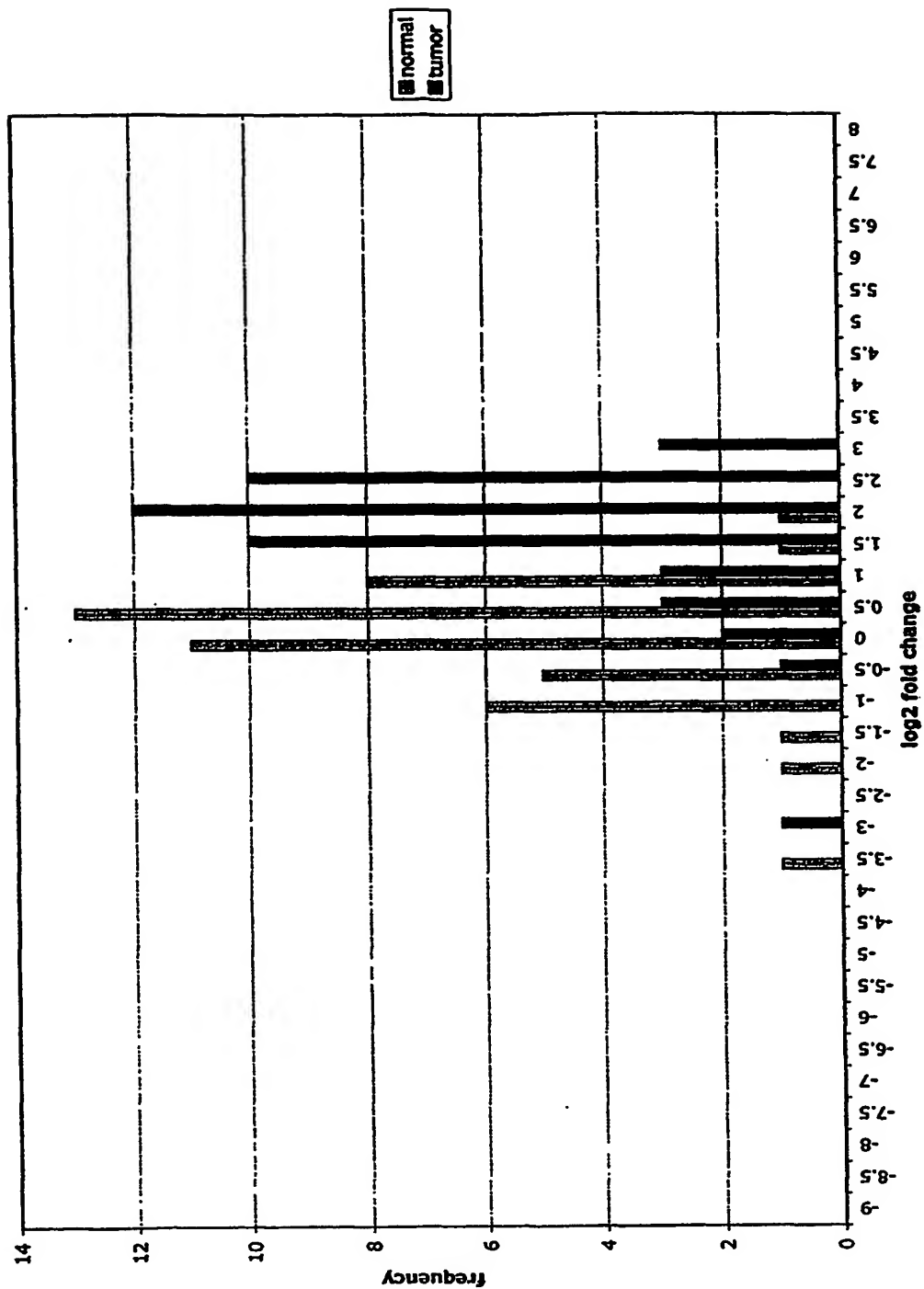


Figure 5(o)

SFRP2-tumor:median normal log2 fold change

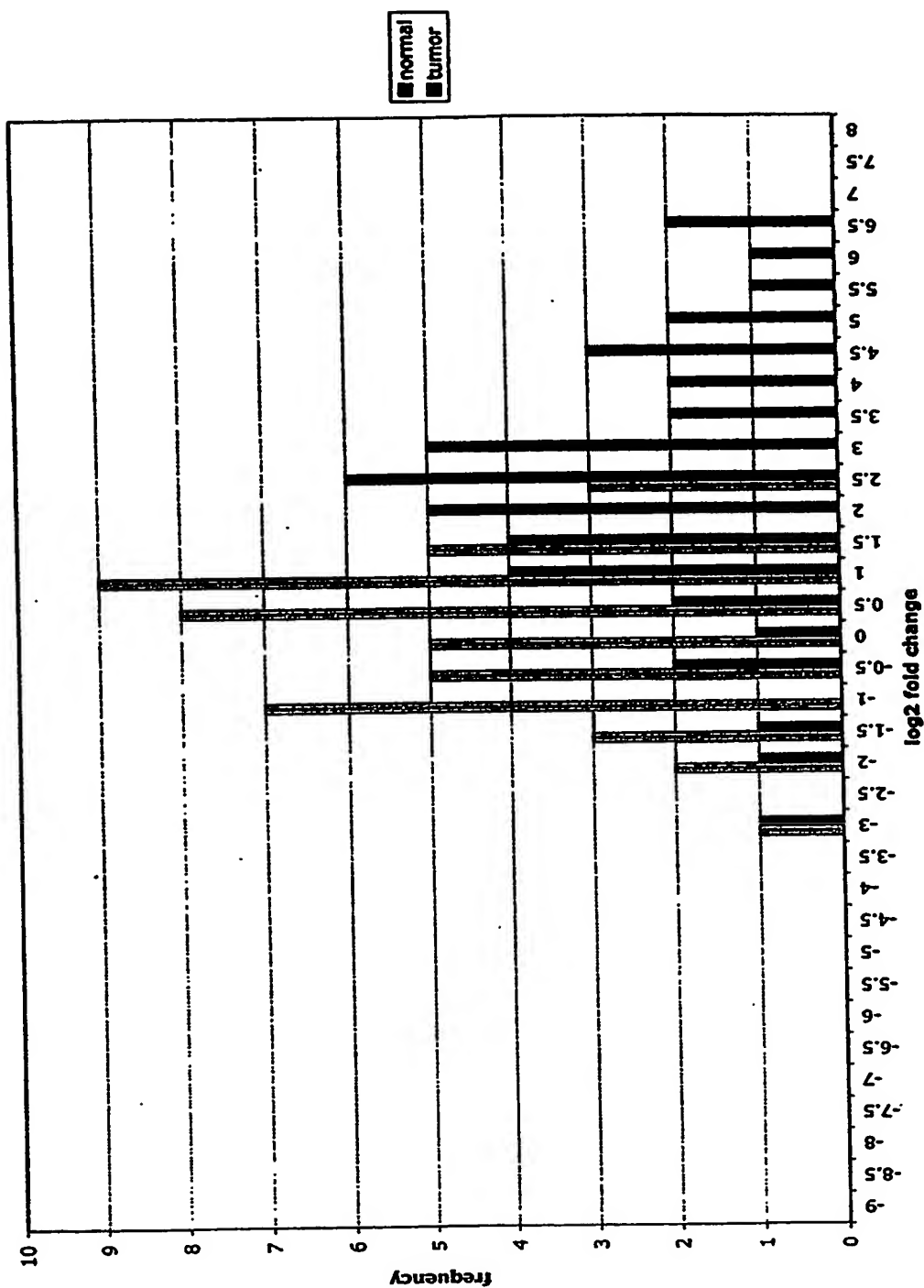


Figure 5(p)

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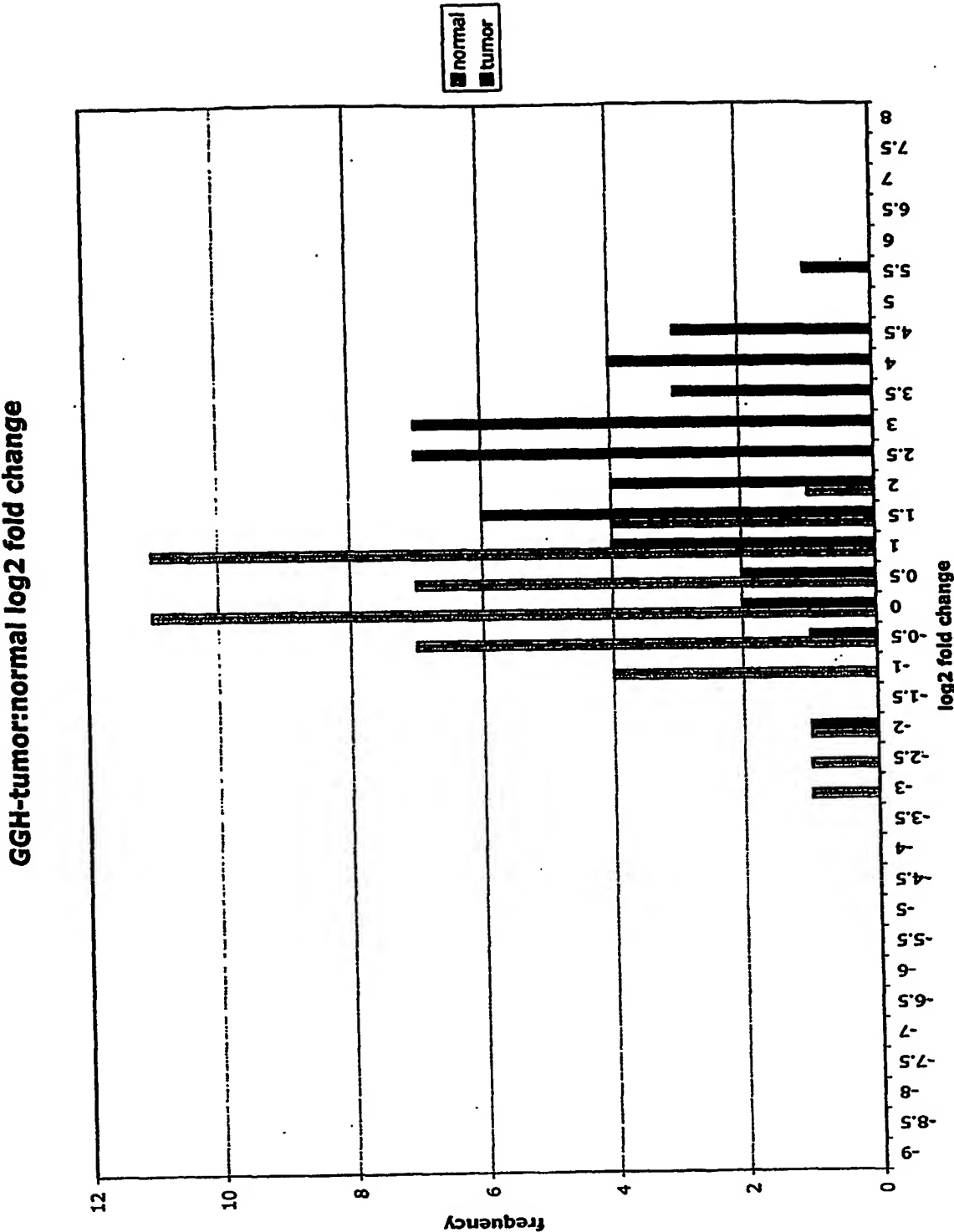


Figure 5(q)

MMP12-tumor:median normal log2 fold changes

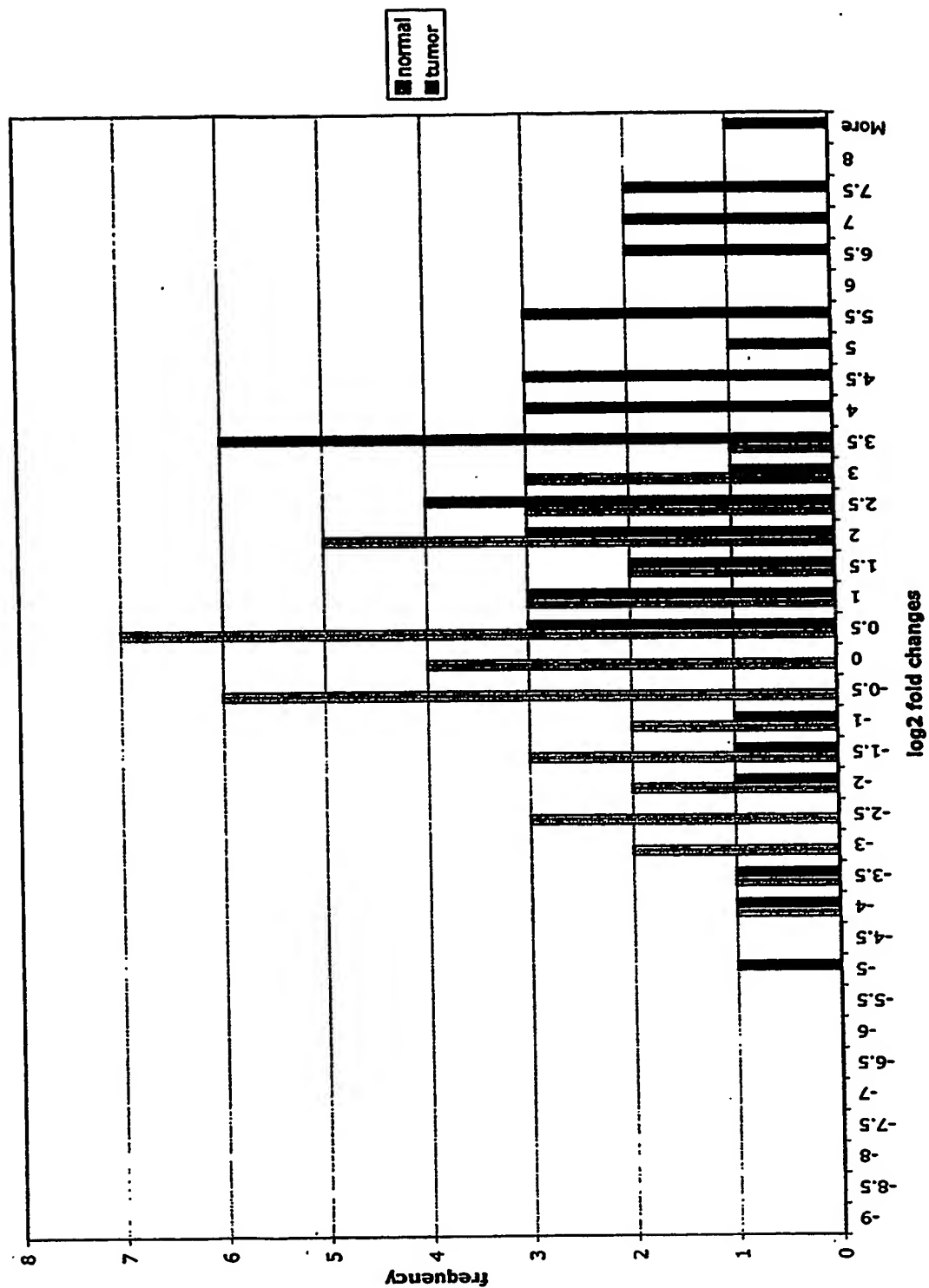


Figure 5(r)

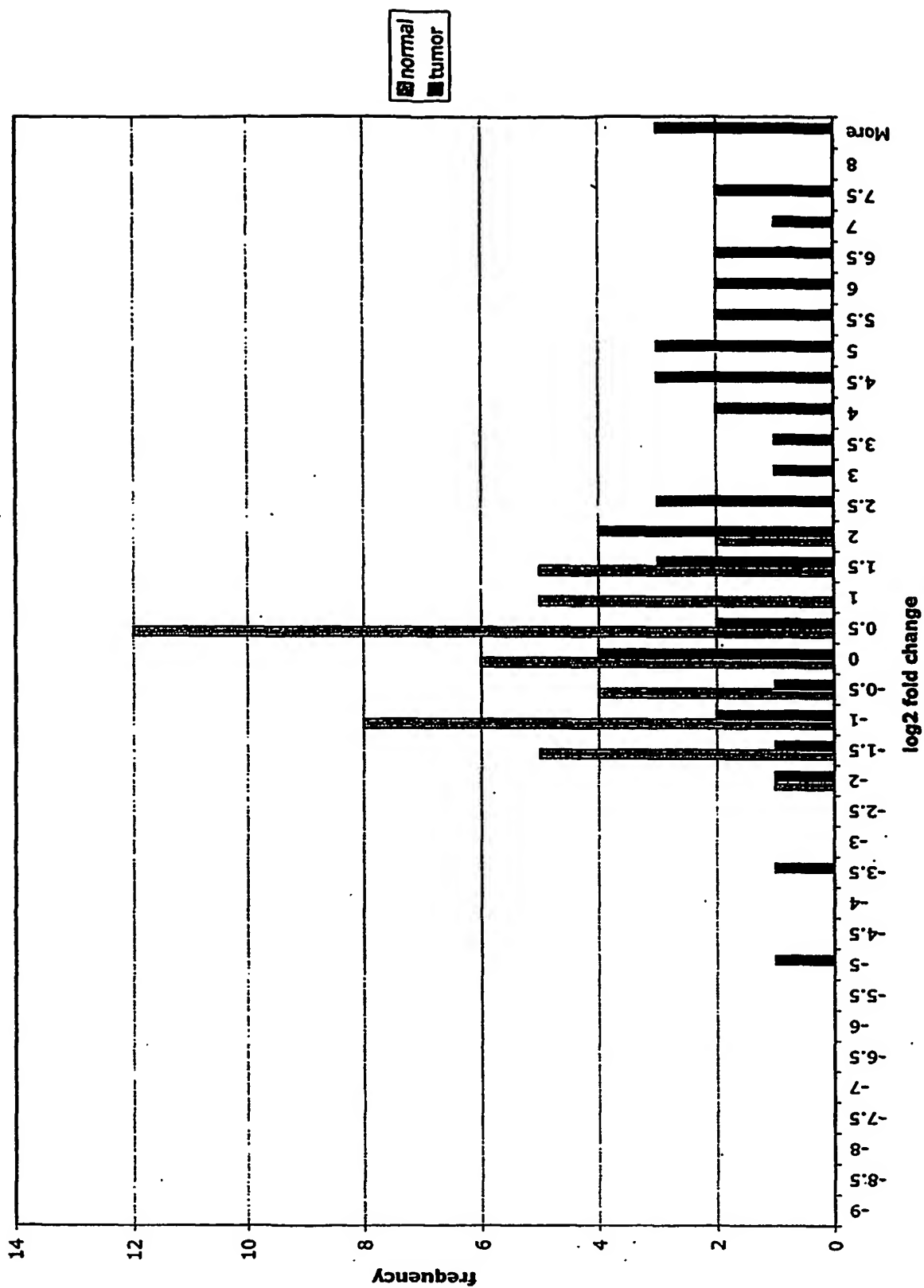


Figure 5(s)

LEPRE1-tumor:median normal log2 fold changes

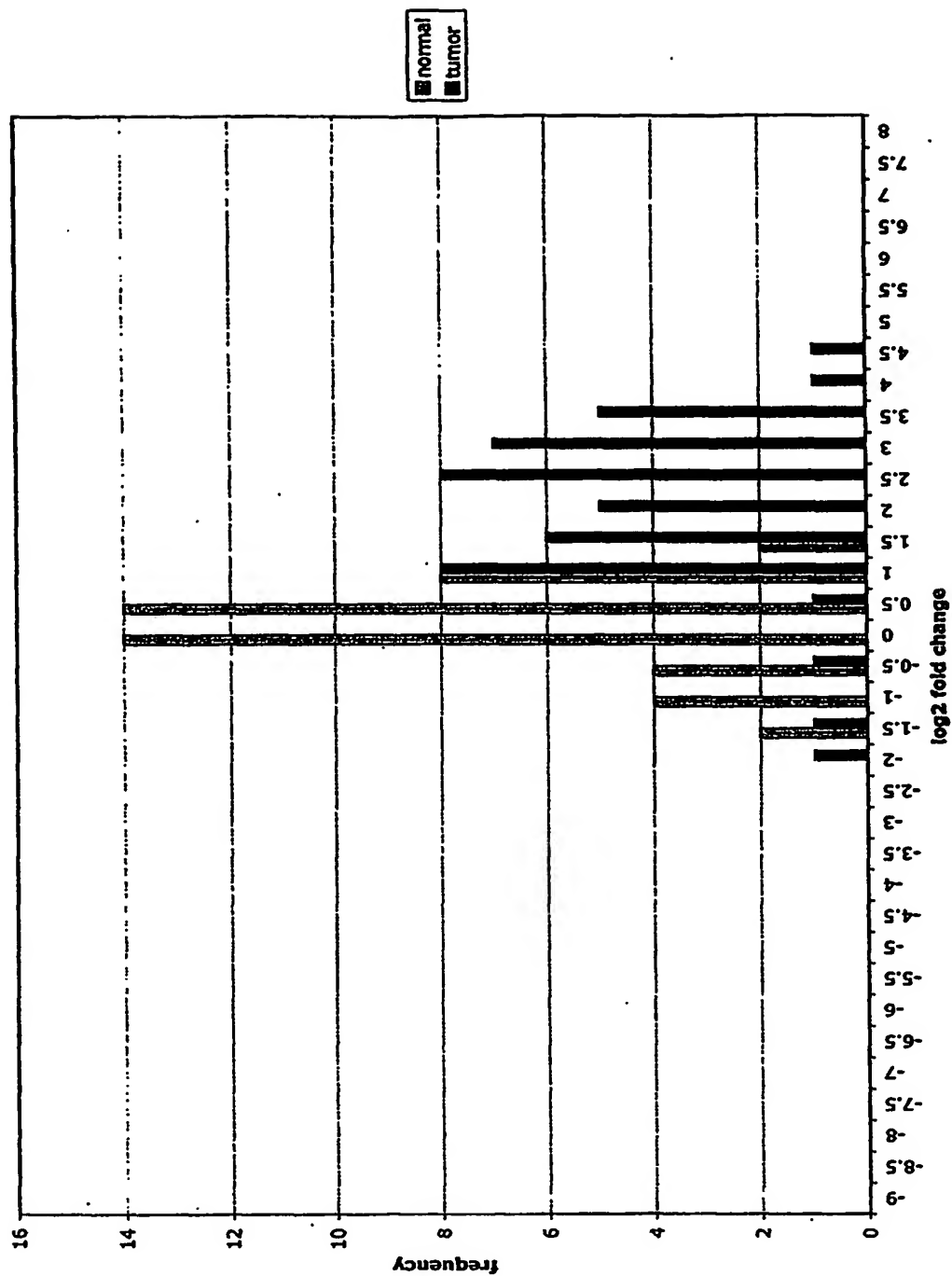


Figure 5(t)

TG-tumor:median normal log2 fold change

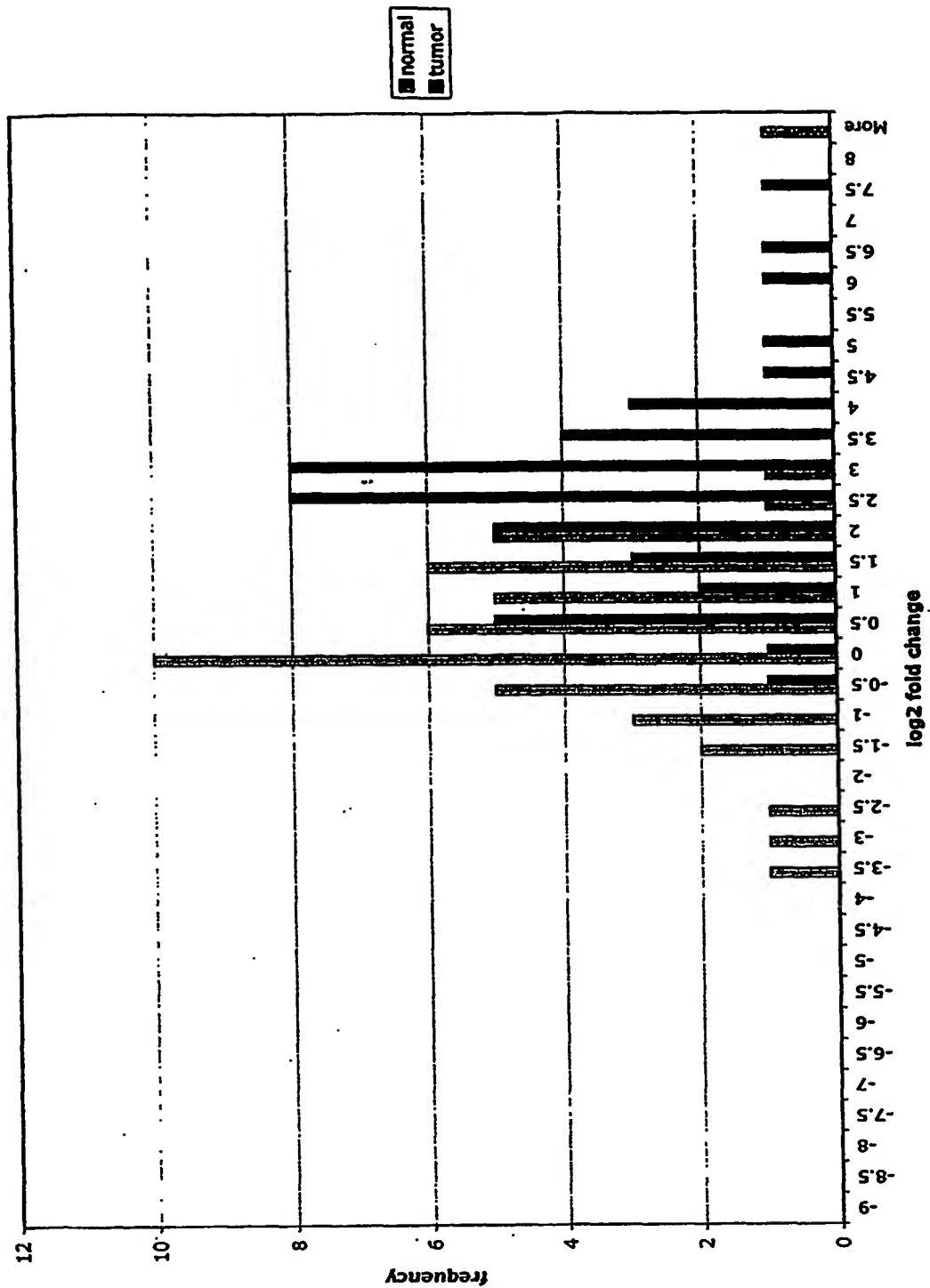


Figure 5(u)

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EFEMP2-tumor:median normal log2 fold change

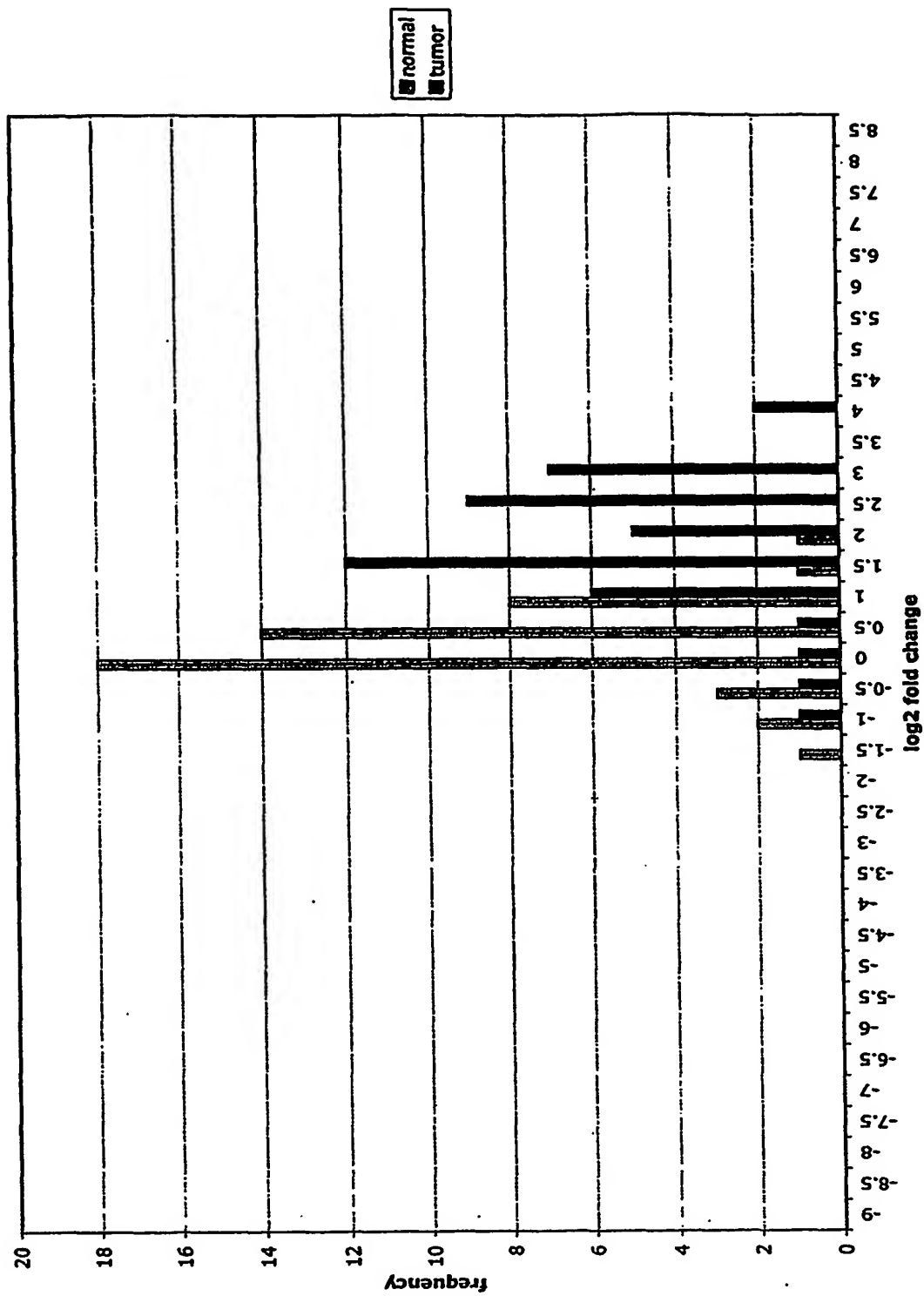


Figure 5(v)

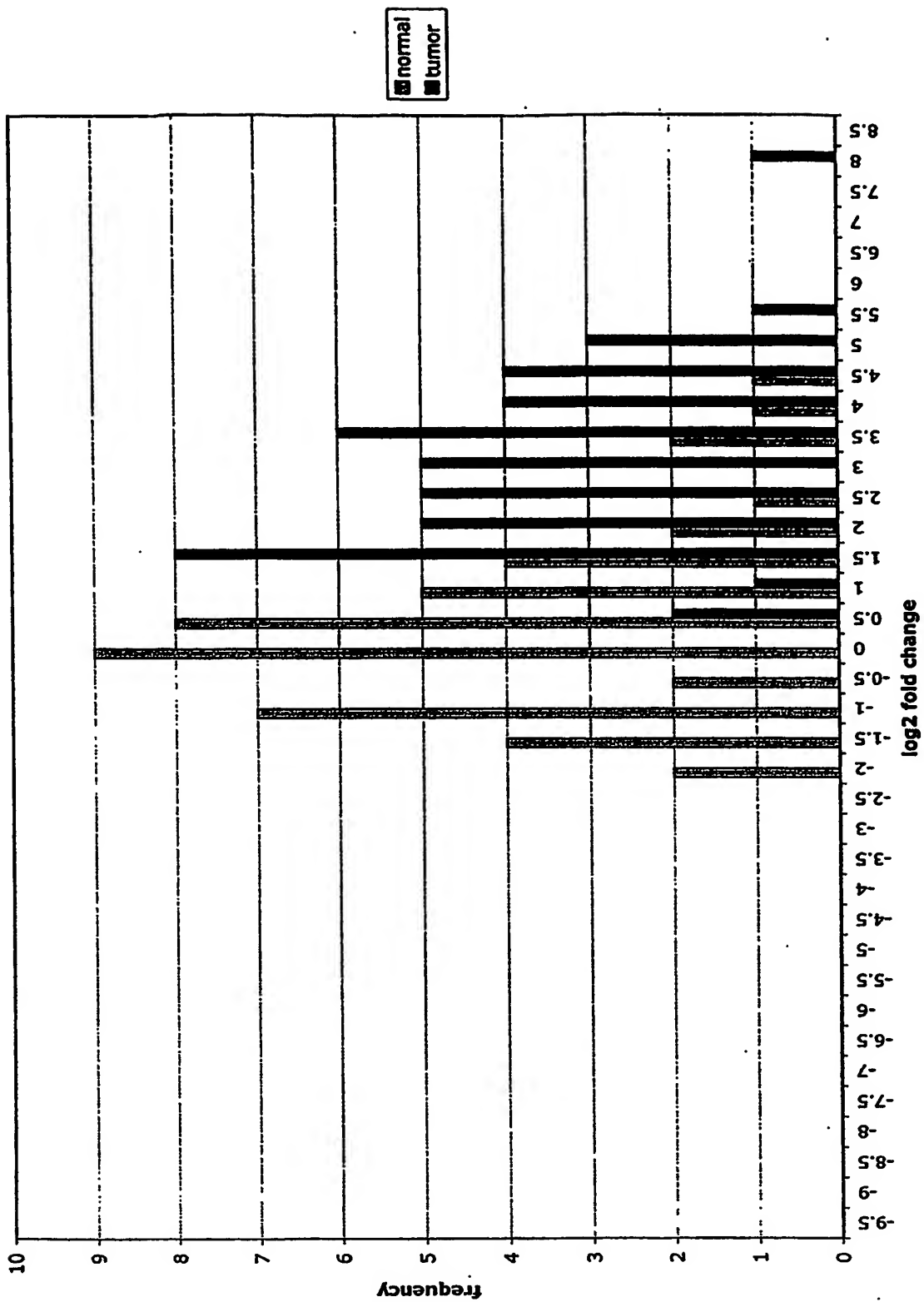


Figure 5(w)

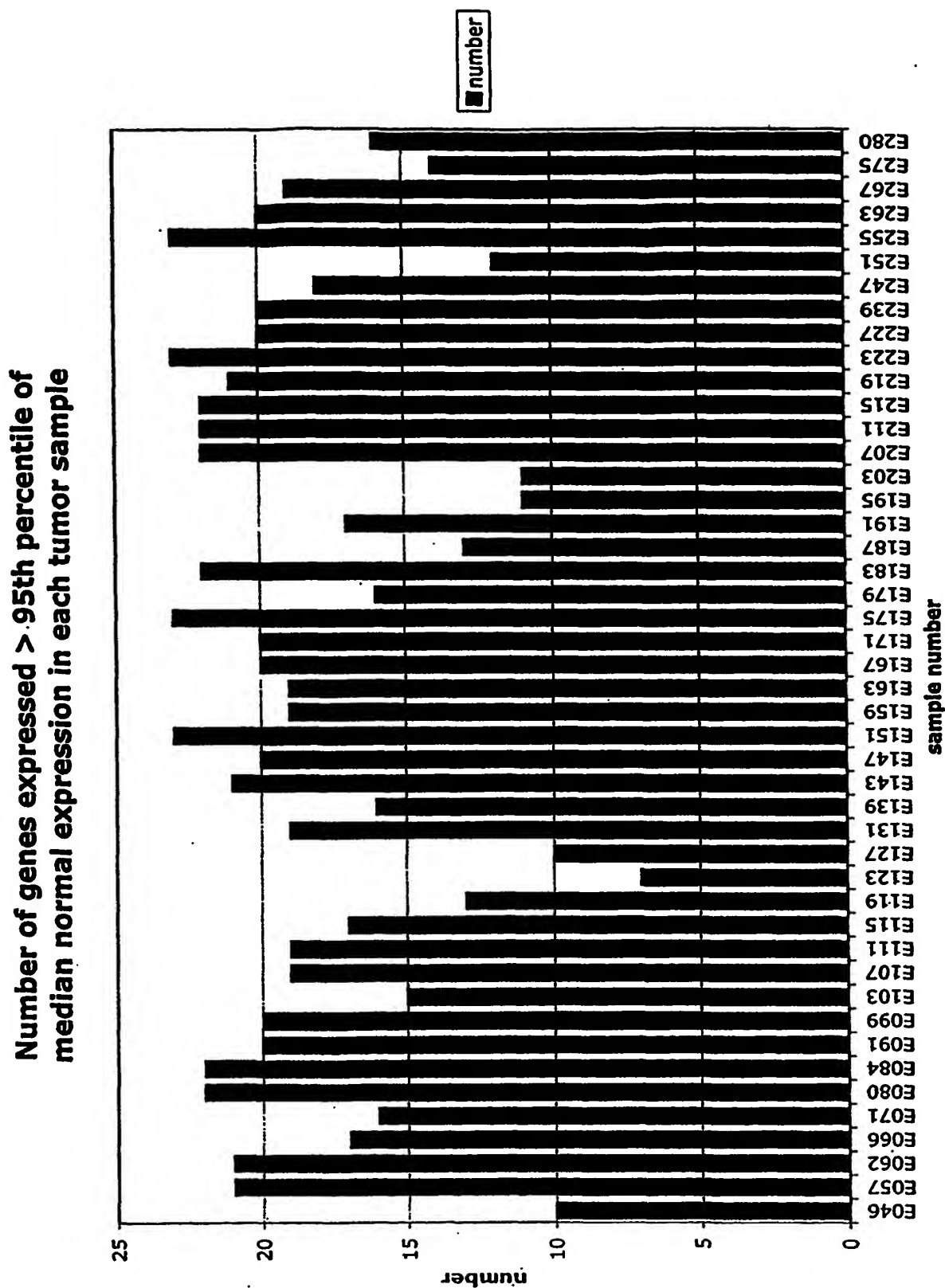


Figure 6

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Fig.7a Relative expression of markers in tumor and normal samples compared to CEA

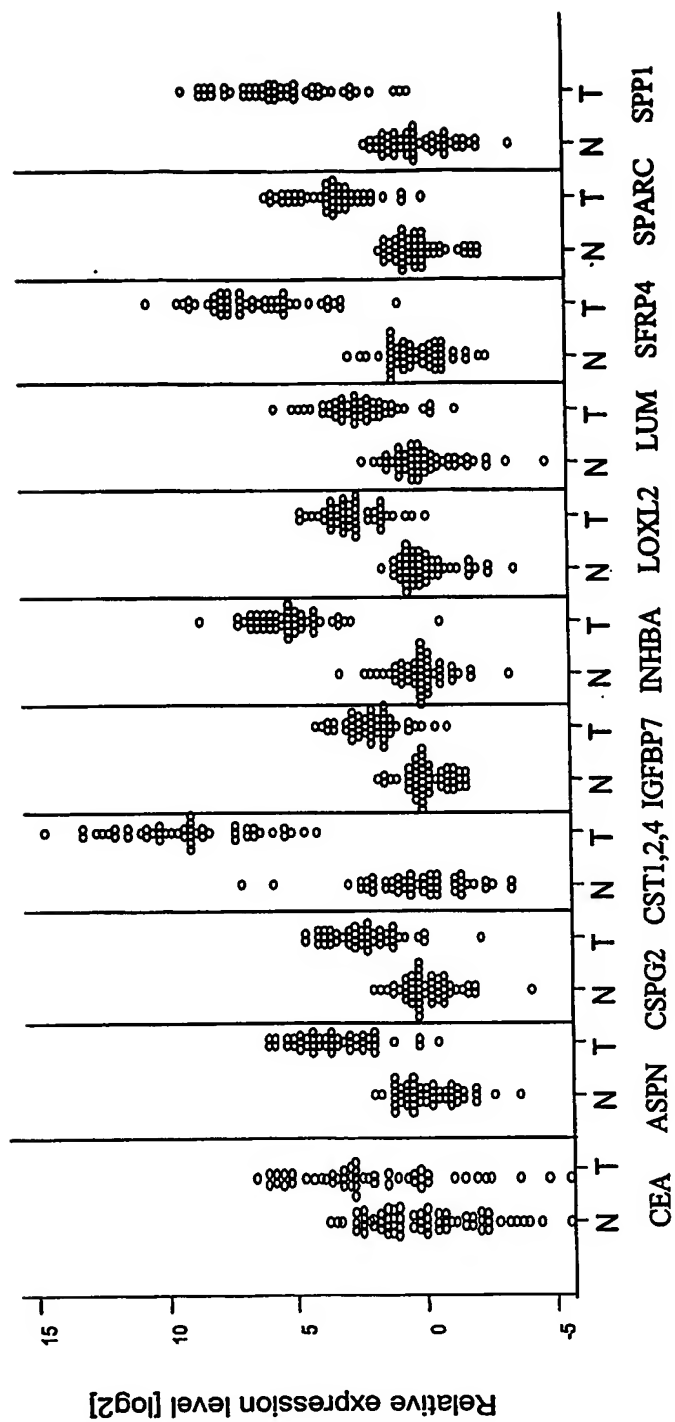
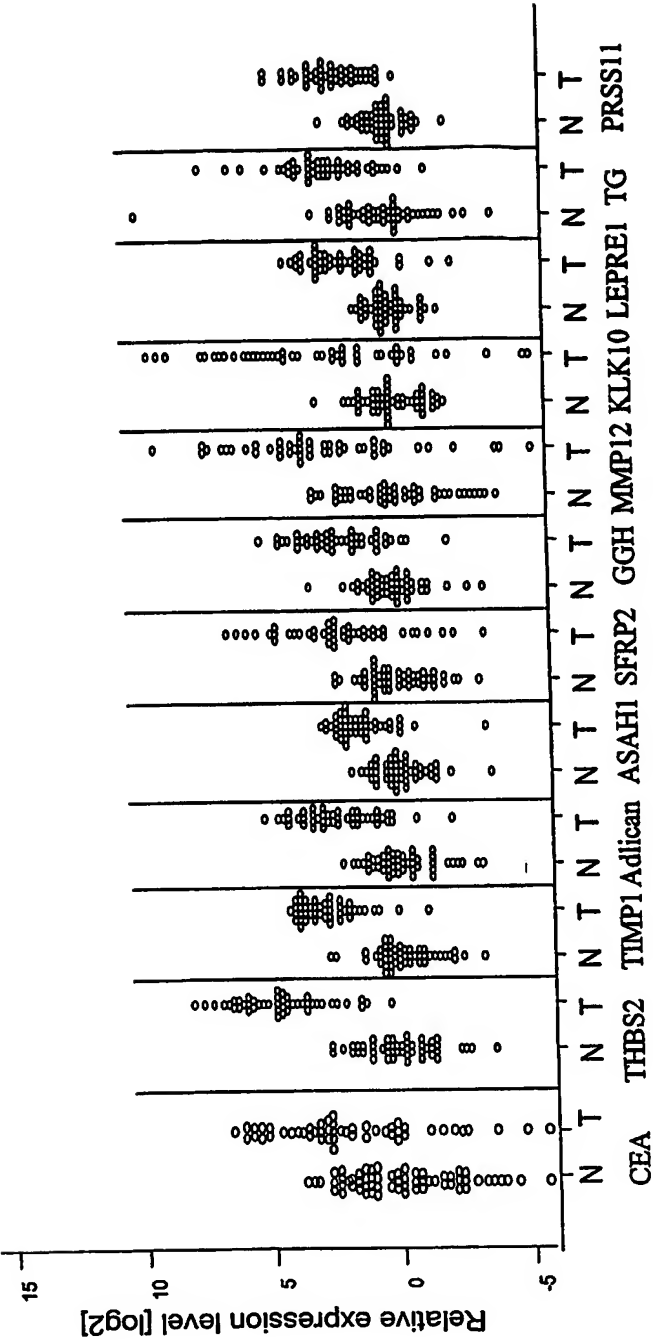


Fig. 7b



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Fig. 7c

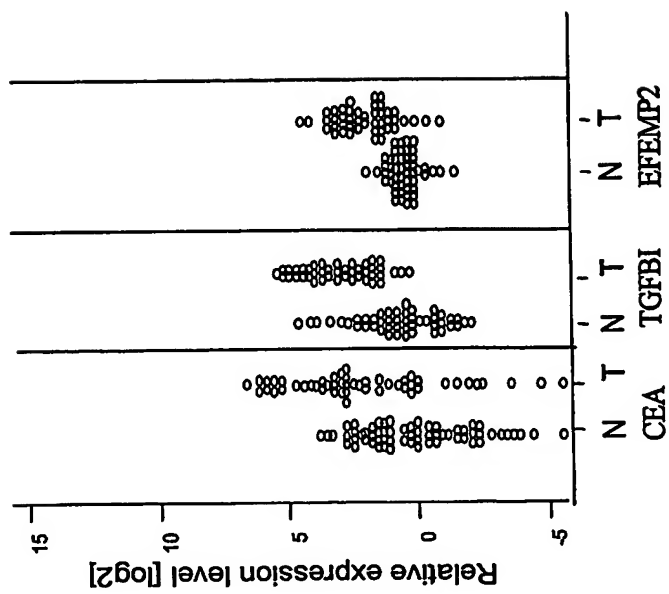


Fig. 8. Quantitative RT-PCR: expression in paired tumor and non-malignant samples of selected gastric cancer markers

name	symbol	median T:N fold change	maximum T:N fold change	% tumor samples with expression > paired non-malignant sample
adipon		5	146	88
asporin (lr class 1)	ASPN	11	198	100
chondroitin sulfate proteoglycan 2 (versican)	CSPG2	5	68	93
cystatins SN, SA & S	CST1, 2, 4	498	11911	100
egf-containing fibulin-like extracellular matrix protein 2	EFEMP2	3	17	93
gamma-glutamyl hydrolase	GGH	4	34	83
inhibin beta A chain	INHBA	27	630	95
insulin-like growth factor binding protein 7	IGFBP7	5	38	93
kalikrein 10	KLK10	7	519	78
leucine proline-enriched proteoglycan 1 (leprecan 1)	LEPRE1	4	23	85
lumican	LUM	5	68	90
lysyl oxidase-like 2	LOXL2	7	53	95
matrix metalloproteinase 12	MMP12	9	468	85
metalloproteinase inhibitor 1	TIMP1	6	103	95
n-acylsphingosine amidohydrolase	ASAH1	3	15	88
osteopontin	SPP1	36	626	98
secreted frizzled-related protein 2	SFRP2	5	48	83
secreted frizzled-related protein 4	SFRP4	54	375	100
secreted protein, acidic, cysteine rich	SPARC	10	66	95
serine protease 11 (IGF binding)	PRSS11	4	63	90
thrombospondin 2	THBS2	23	452	98
thyroglobulin	TG	4	174	93
transforming growth factor B-induced	TGFB1	5	78	95
cell growth regulatory factor with EF-hand domain	GGR11	3	33	75
serine (or cysteine) proteinase inhibitor H1	SERPINH1	10	51	98
matrix metalloproteinase 12	MMP2	2	46	83
proprotein convertase subtilisin/kexin type 5	PCSK5	2	63	80
serine (or cysteine) proteinase inhibitor B5	SERPINH5	5	861	73
transforming growth factor beta	TGFB1	3	16	88
cardioembryonic antrien (CEA)	CEACAM5	3	177	68

Fig. 9a Relative tumor:normal fold changes in paired tumor/normal gastric samples

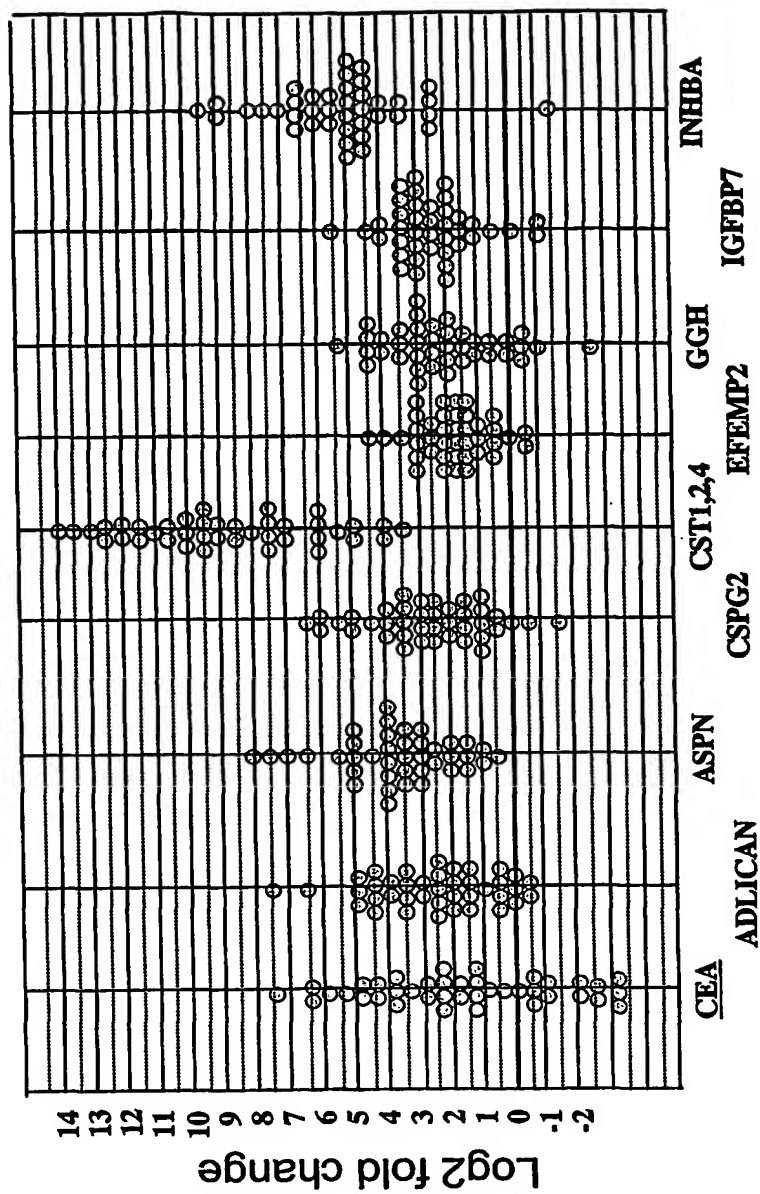
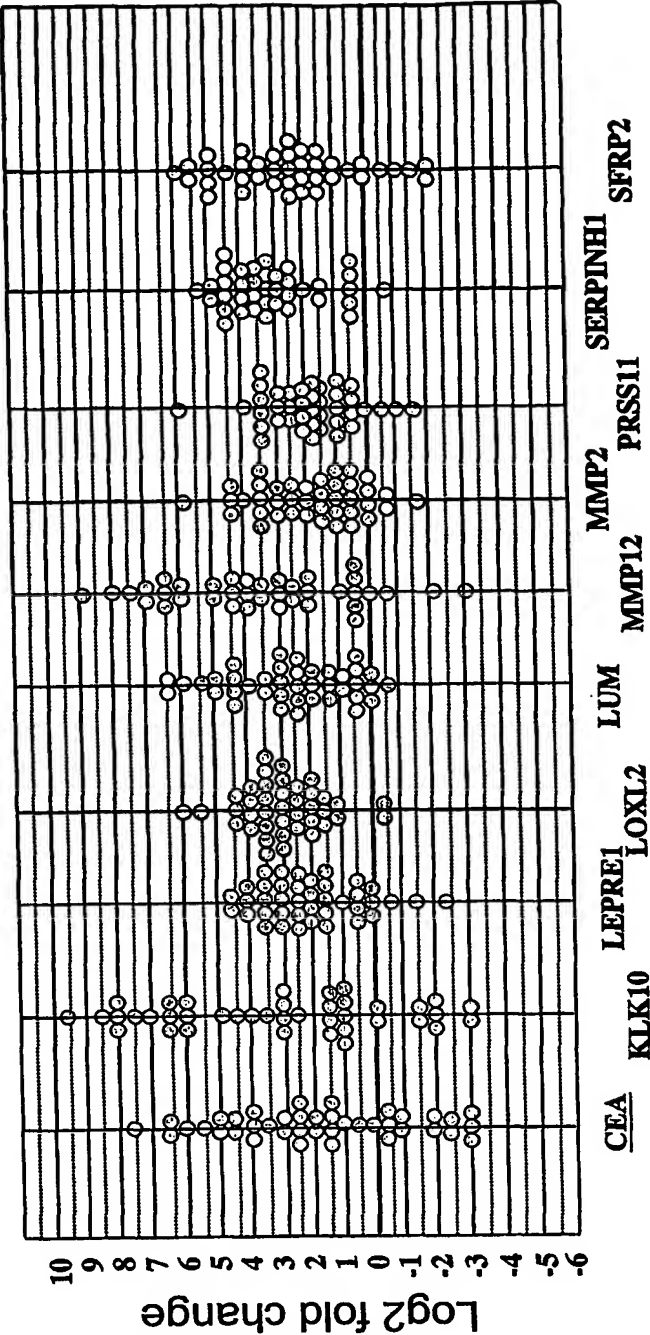


Fig. 9b



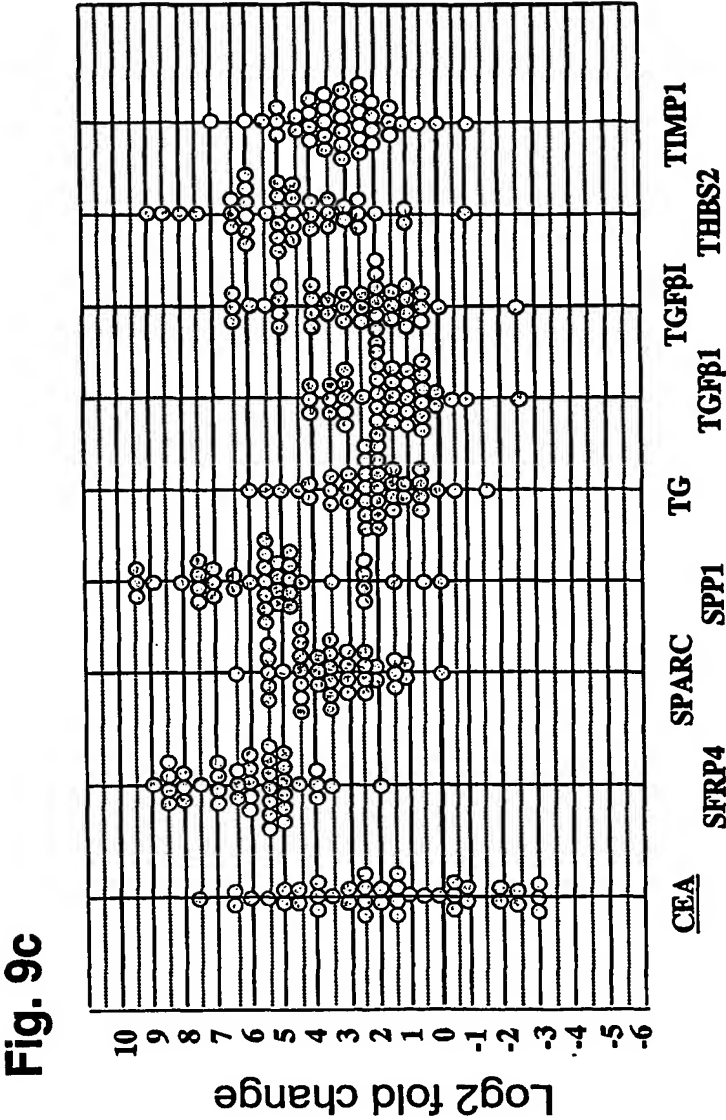


Fig. 9d

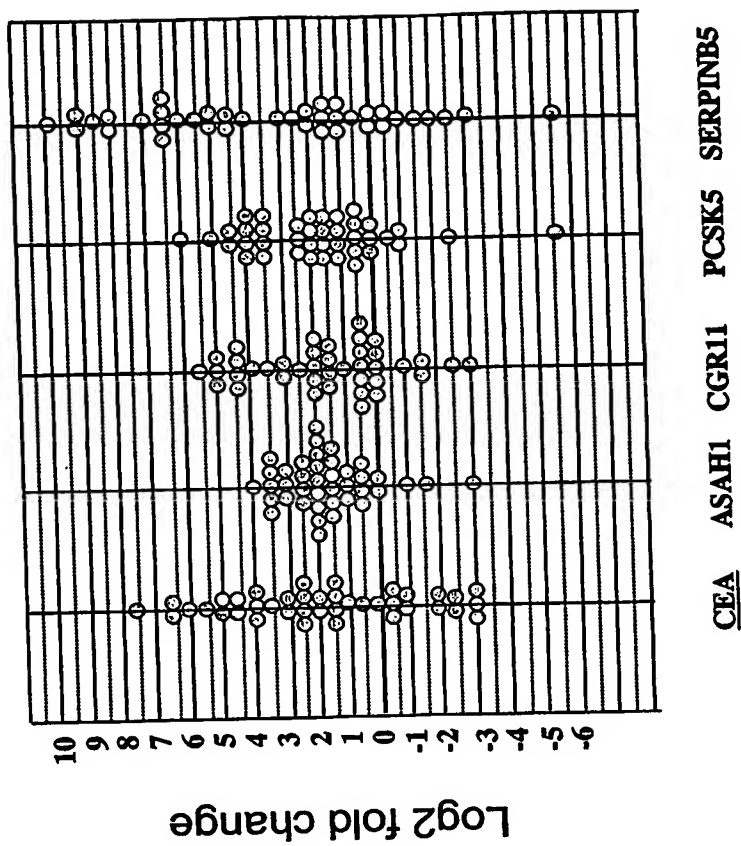


Fig. 10a adllican

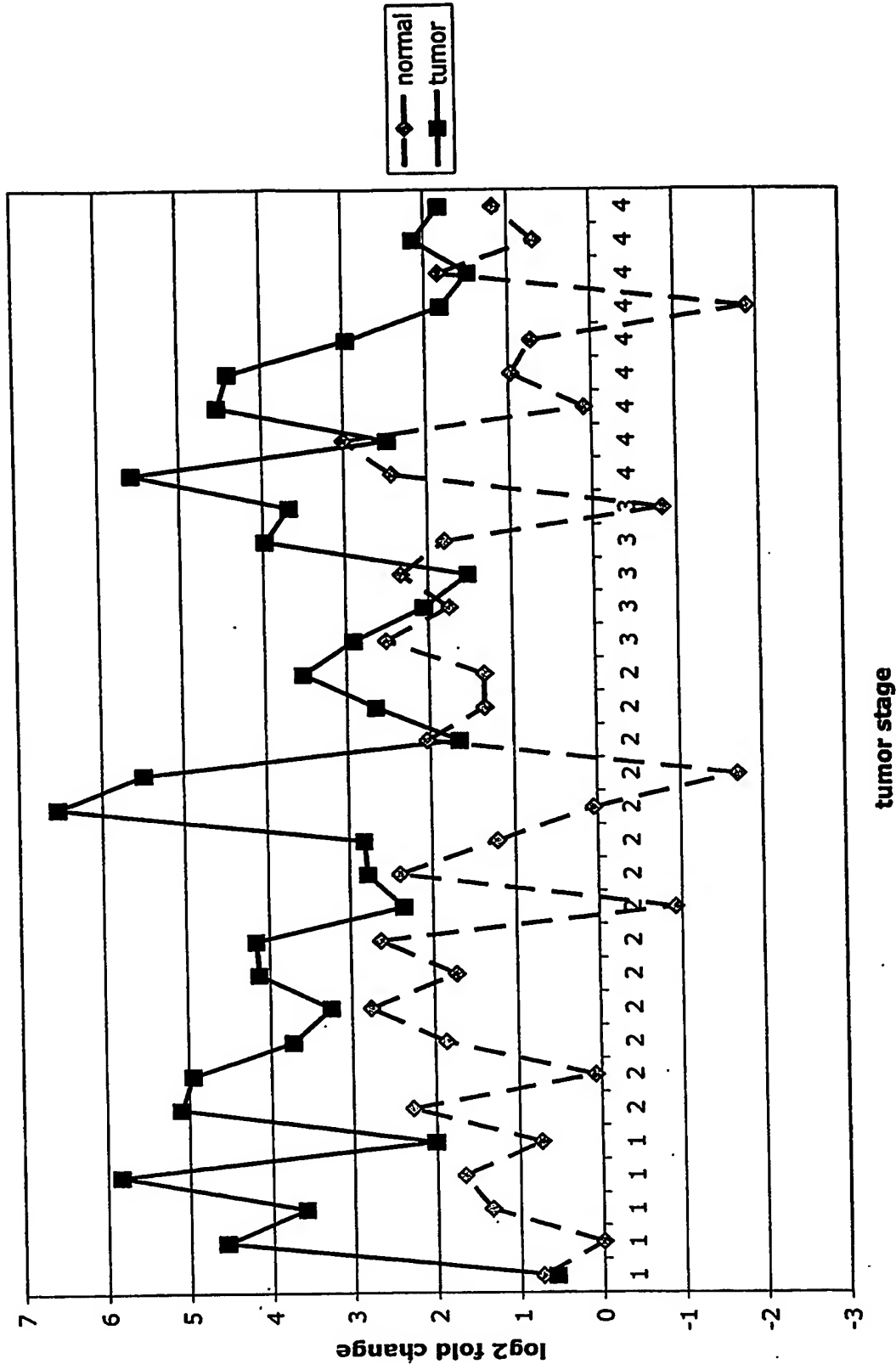


Fig. 10b ASPN

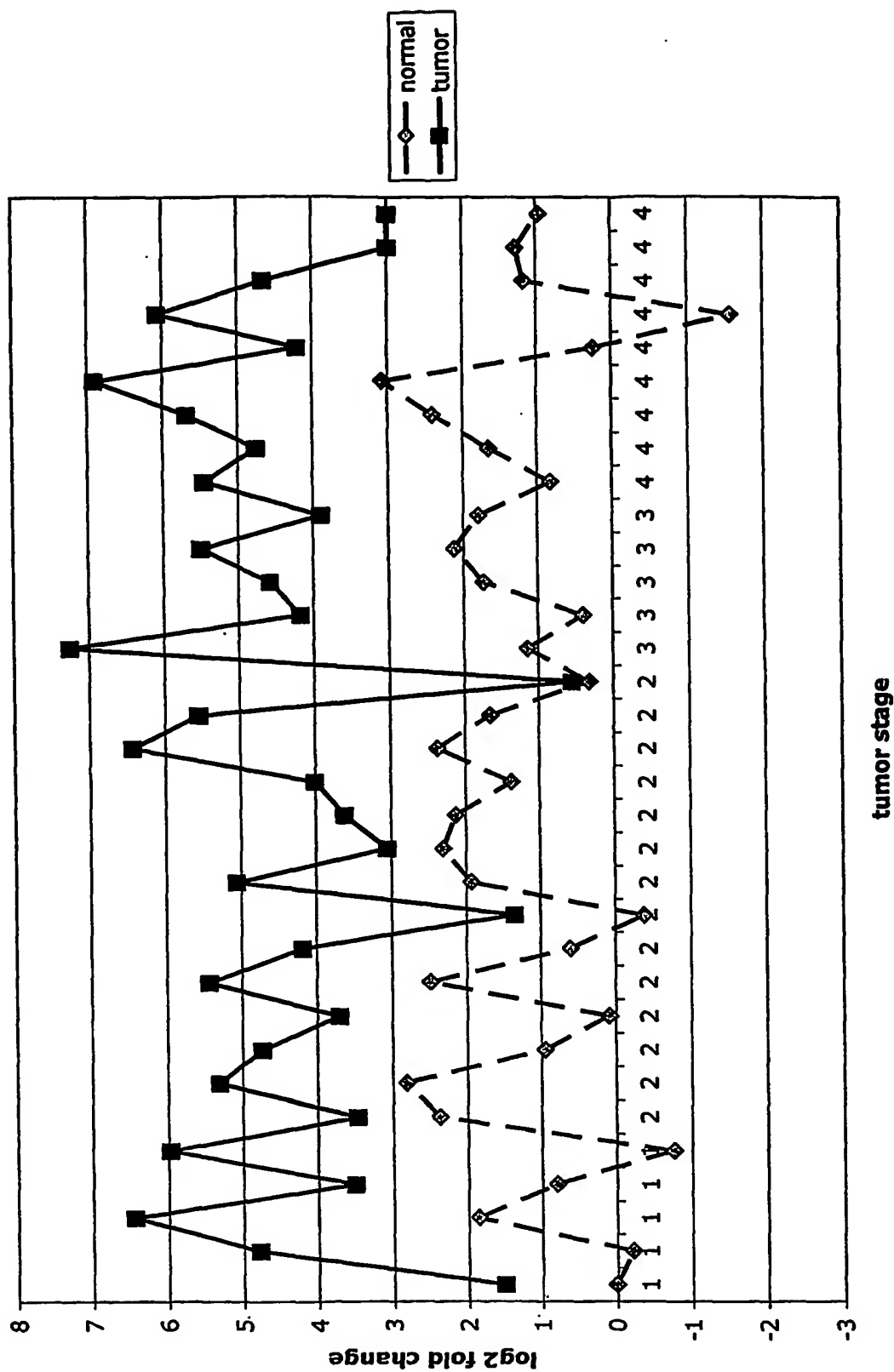


Fig. 10c CSPG2

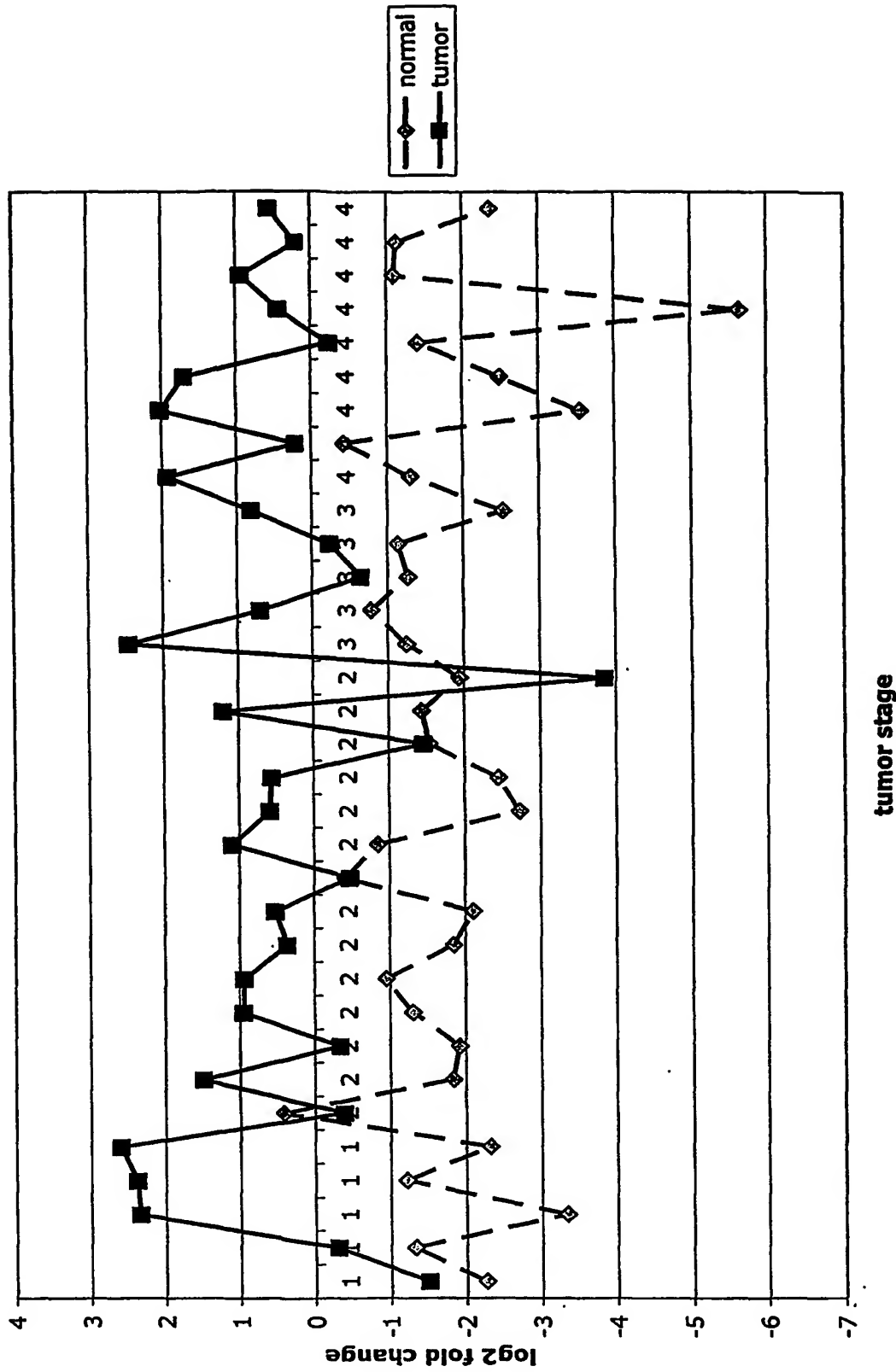


Fig. 10d CST1,2,4

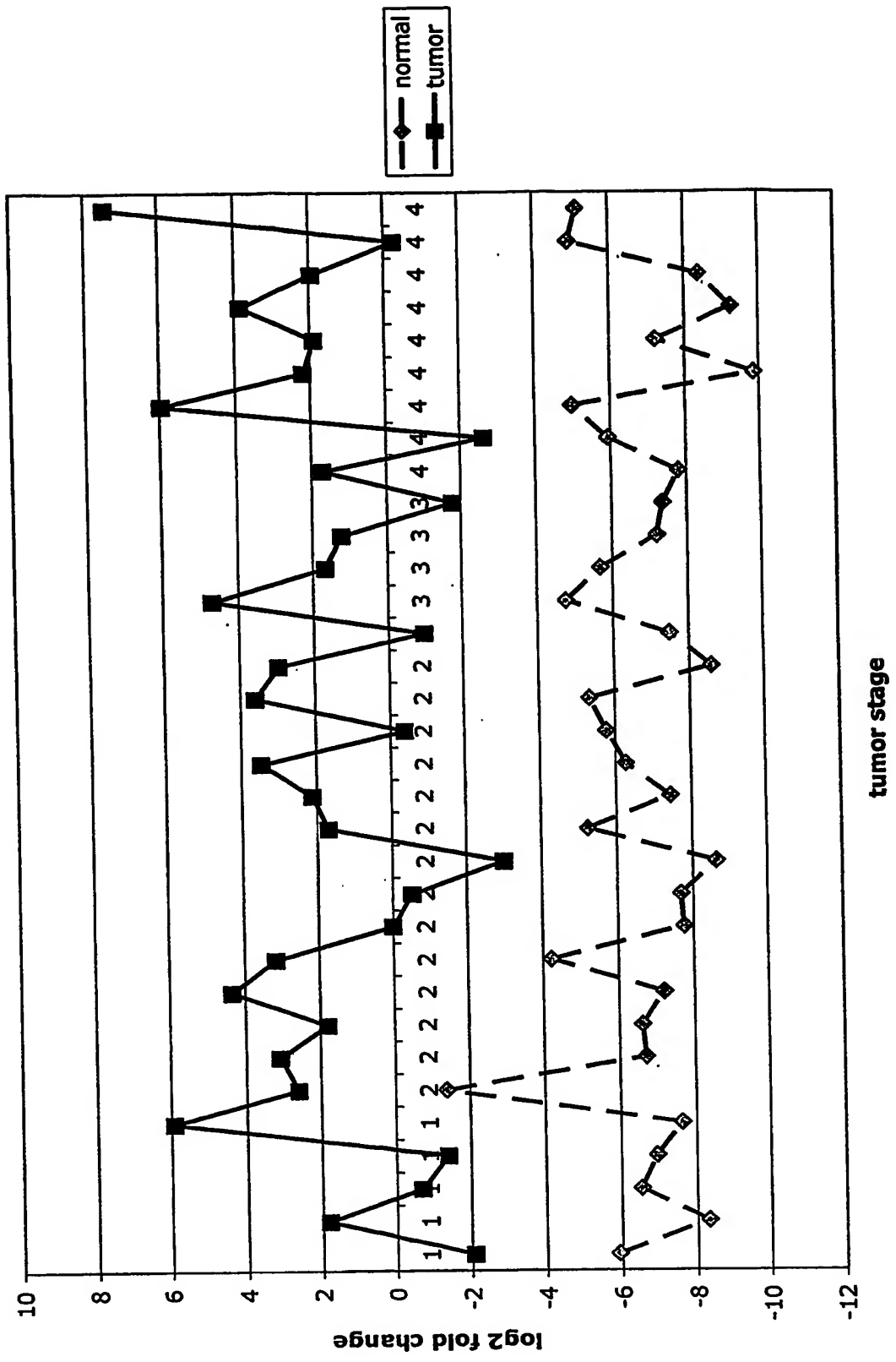


Fig. 10e EFEMP2

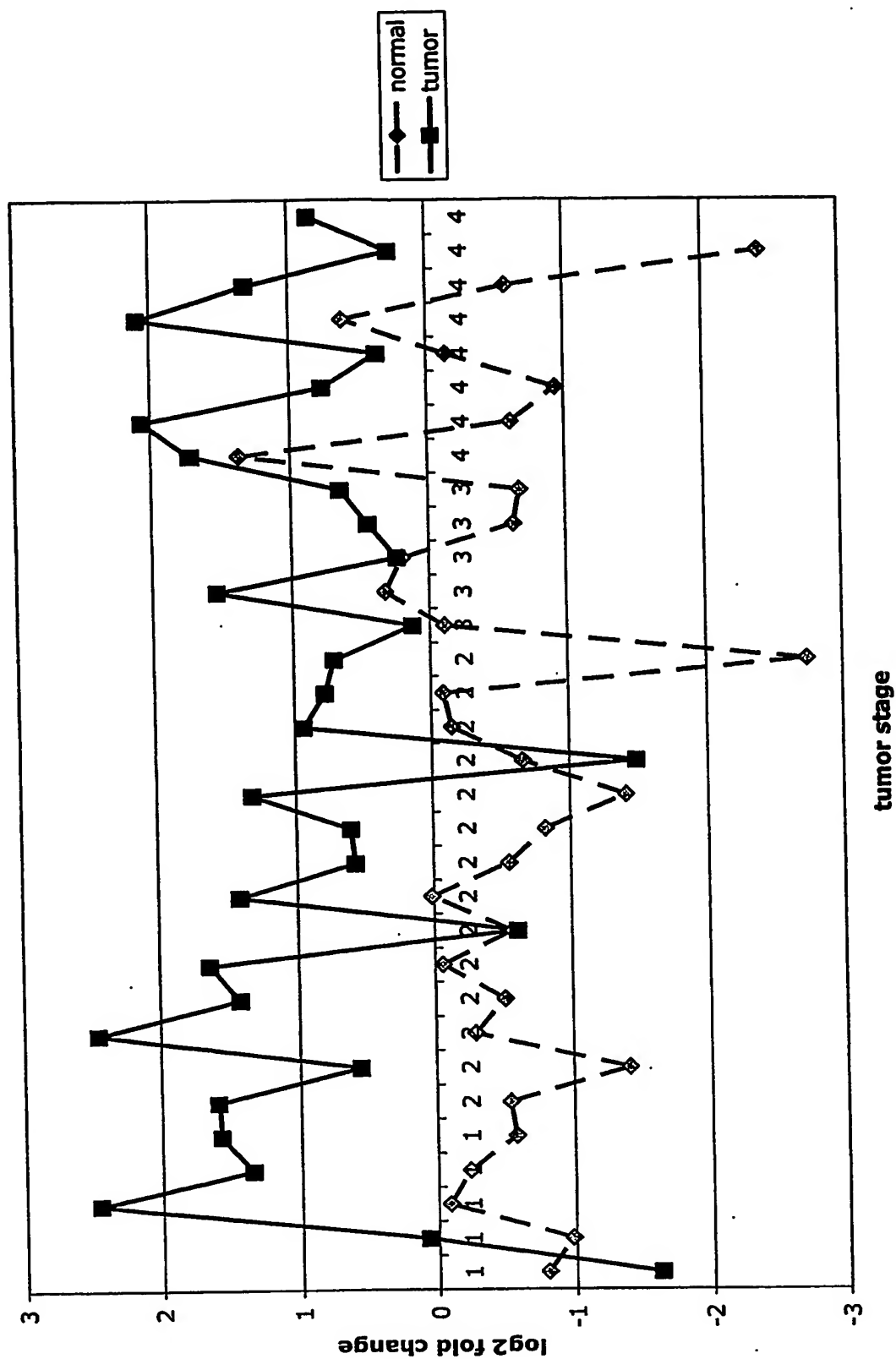
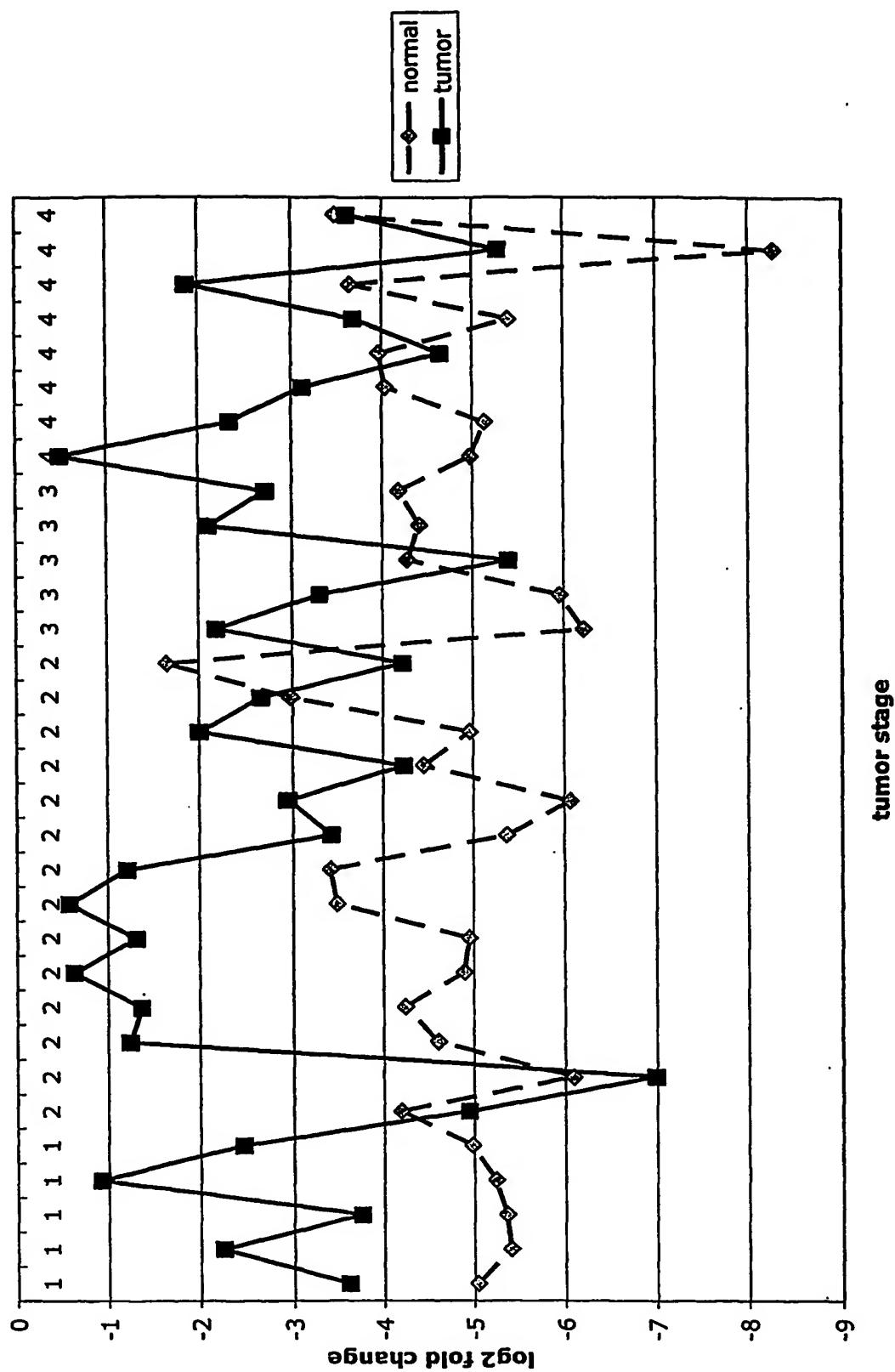


Fig. 10f GGH



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Fig. 10g INHBA

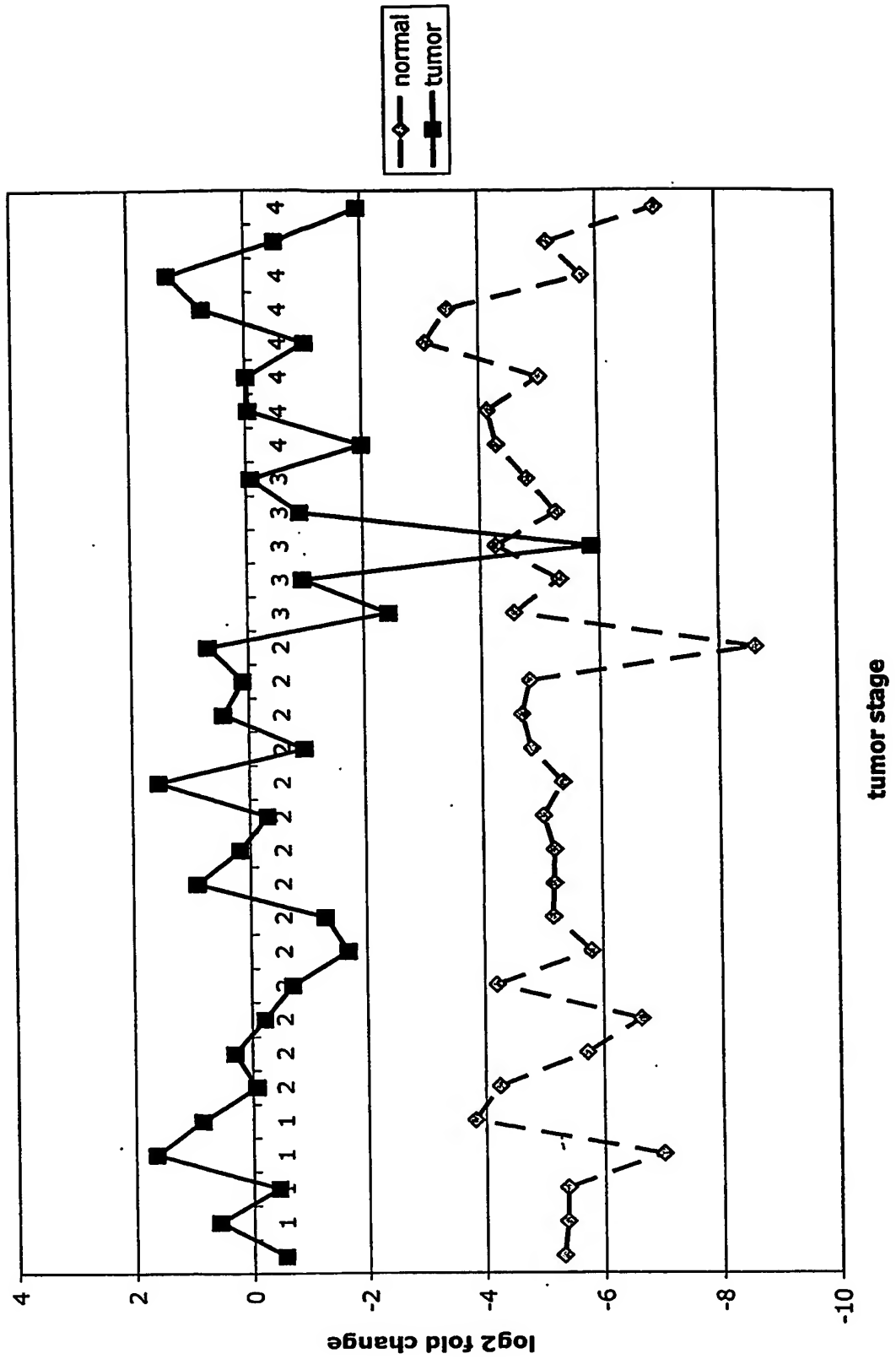


Fig. 10h IGFBP7

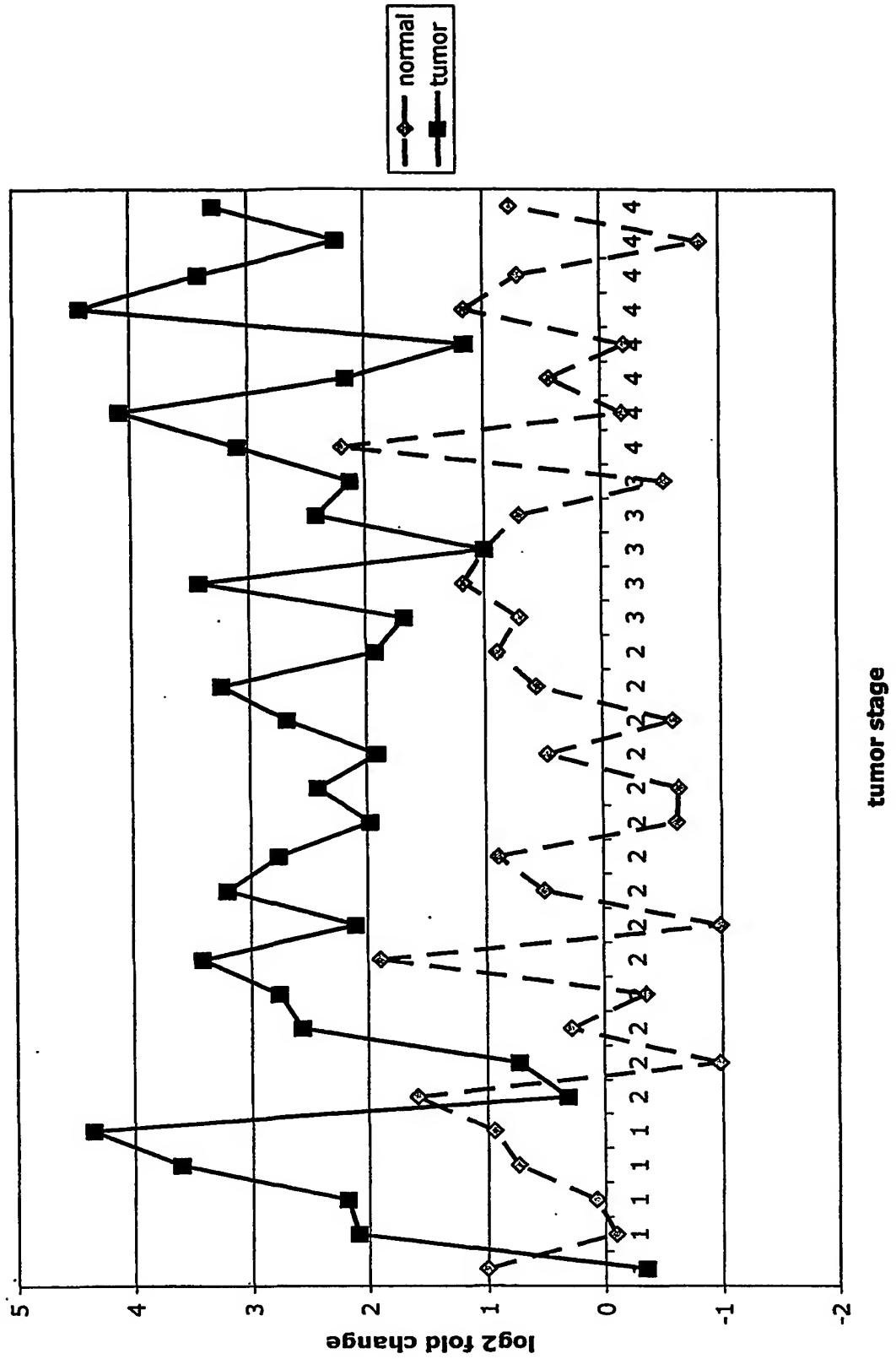
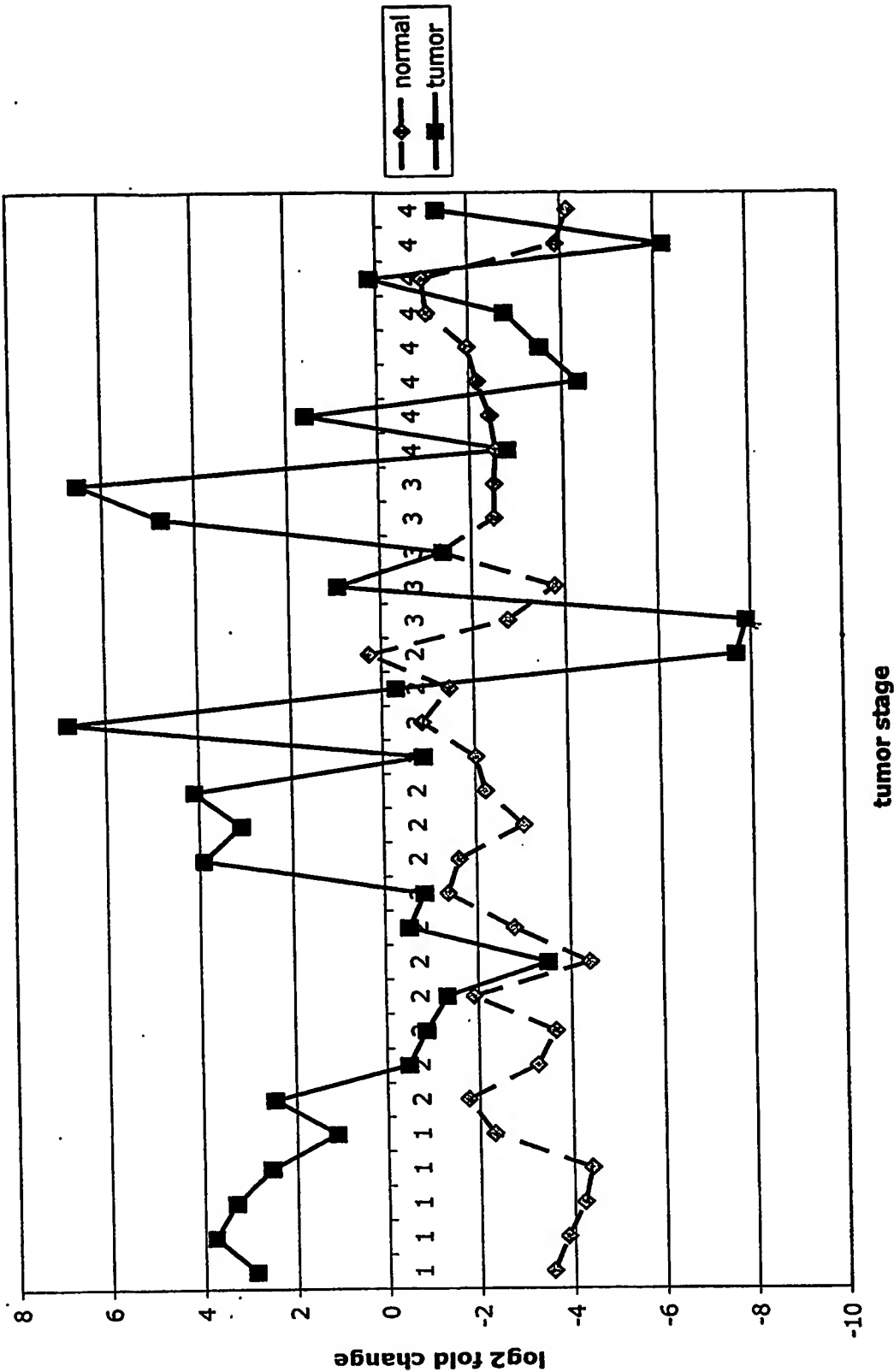


Fig. 10i KLK10



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Fig. 10j LEPRE1

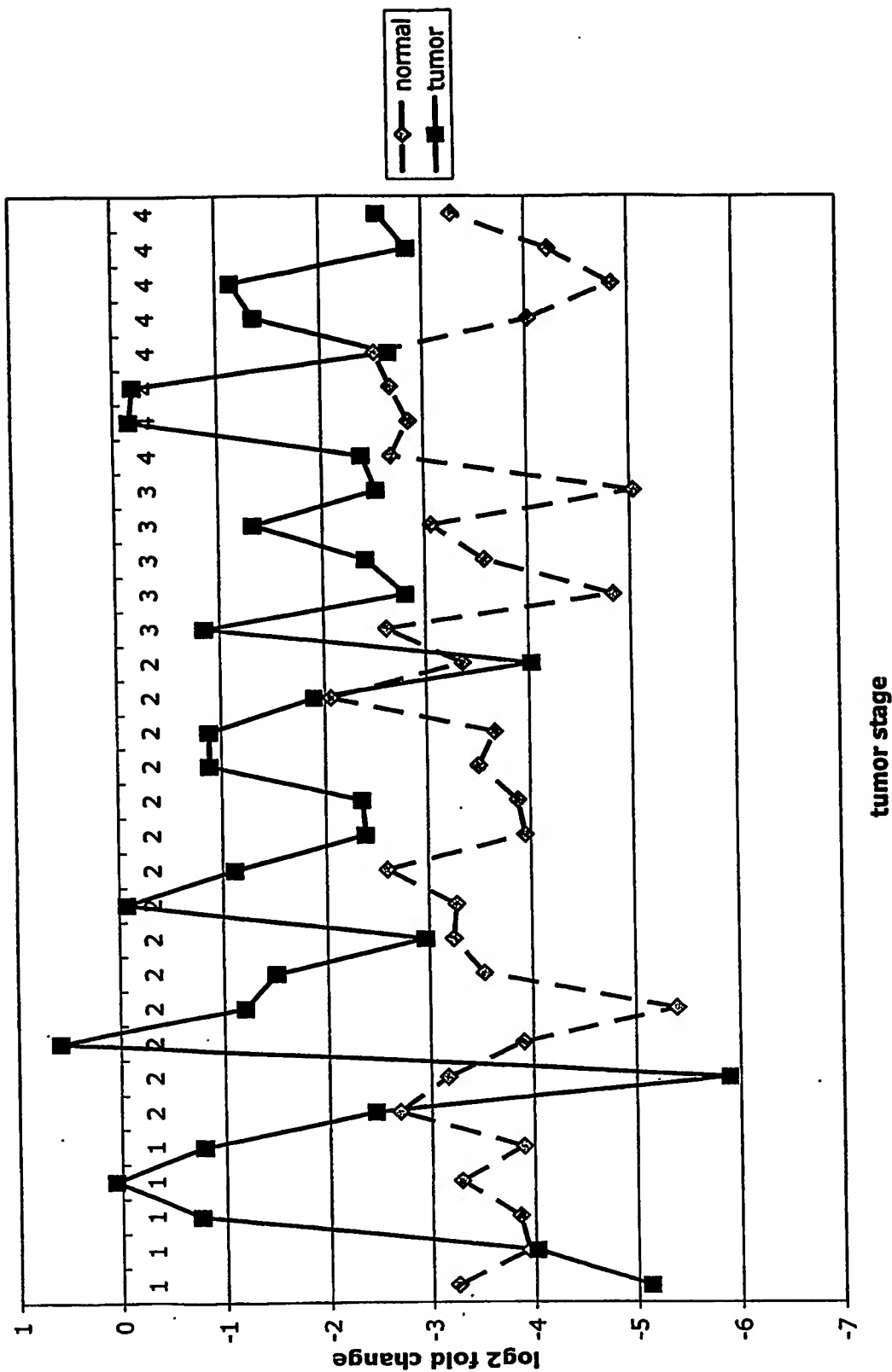


Fig. 10k LUM

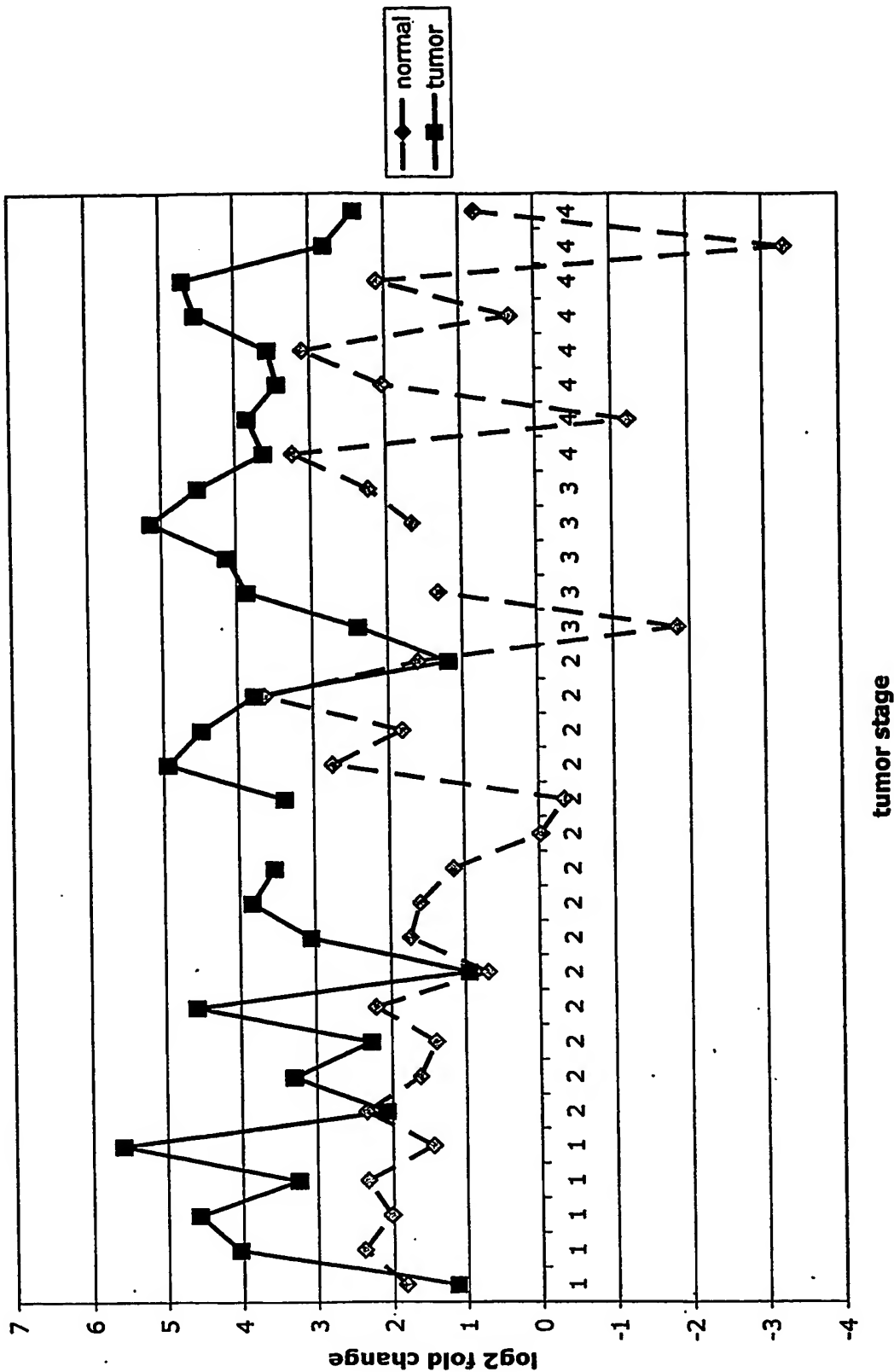


Fig. 10I. LOXL2

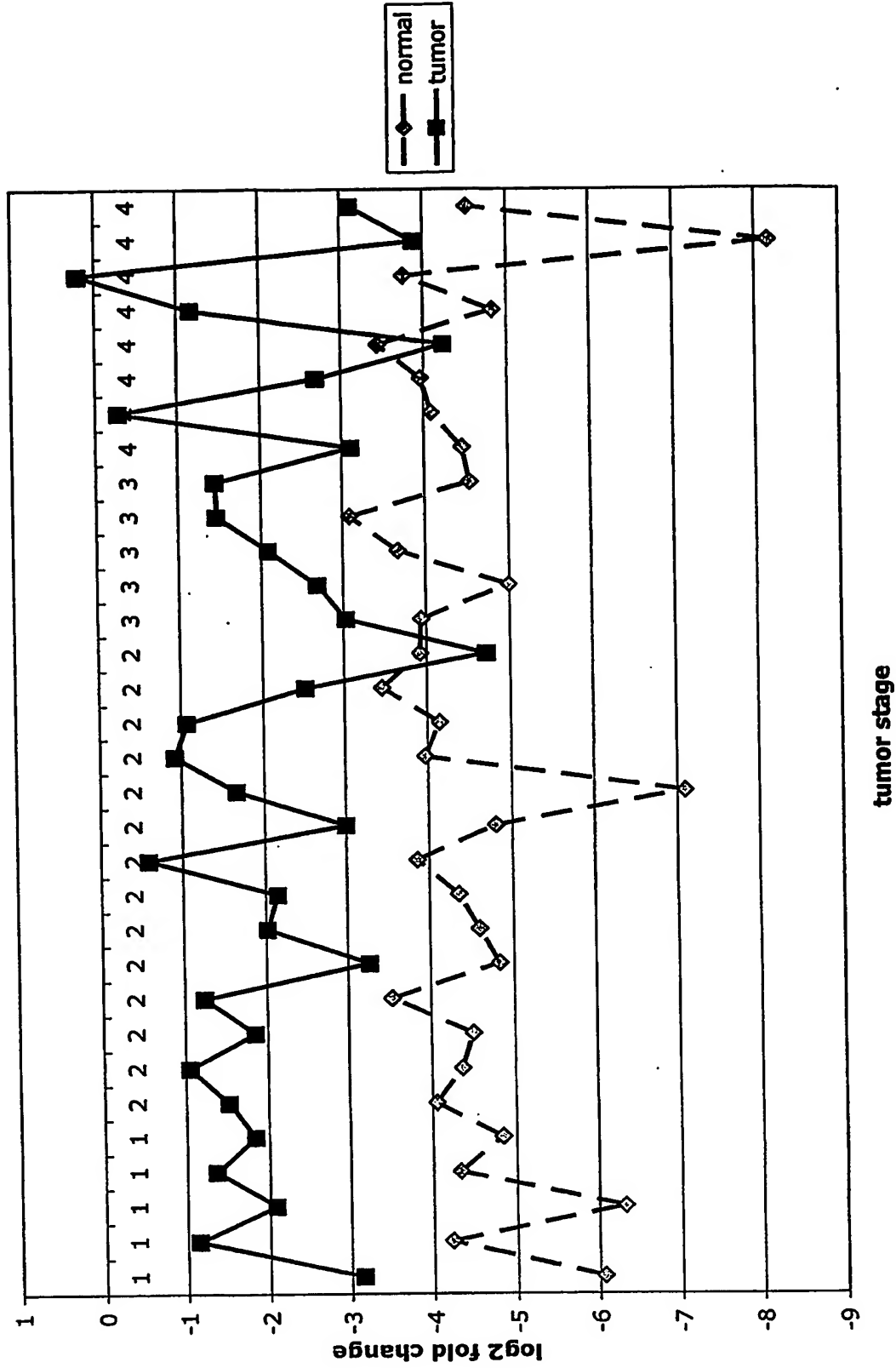


Fig. 10m MMP12

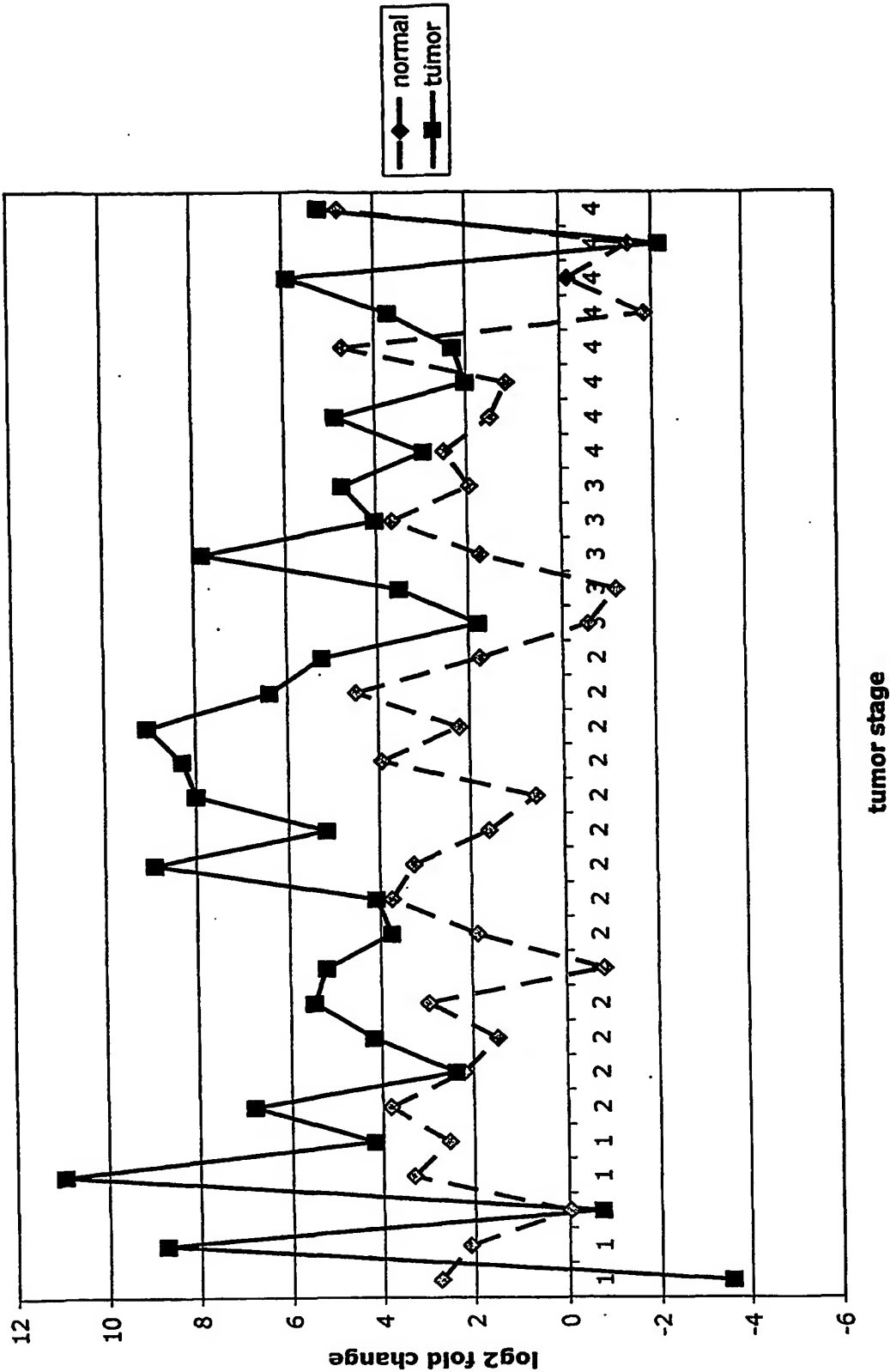


Fig.10n TIMP1

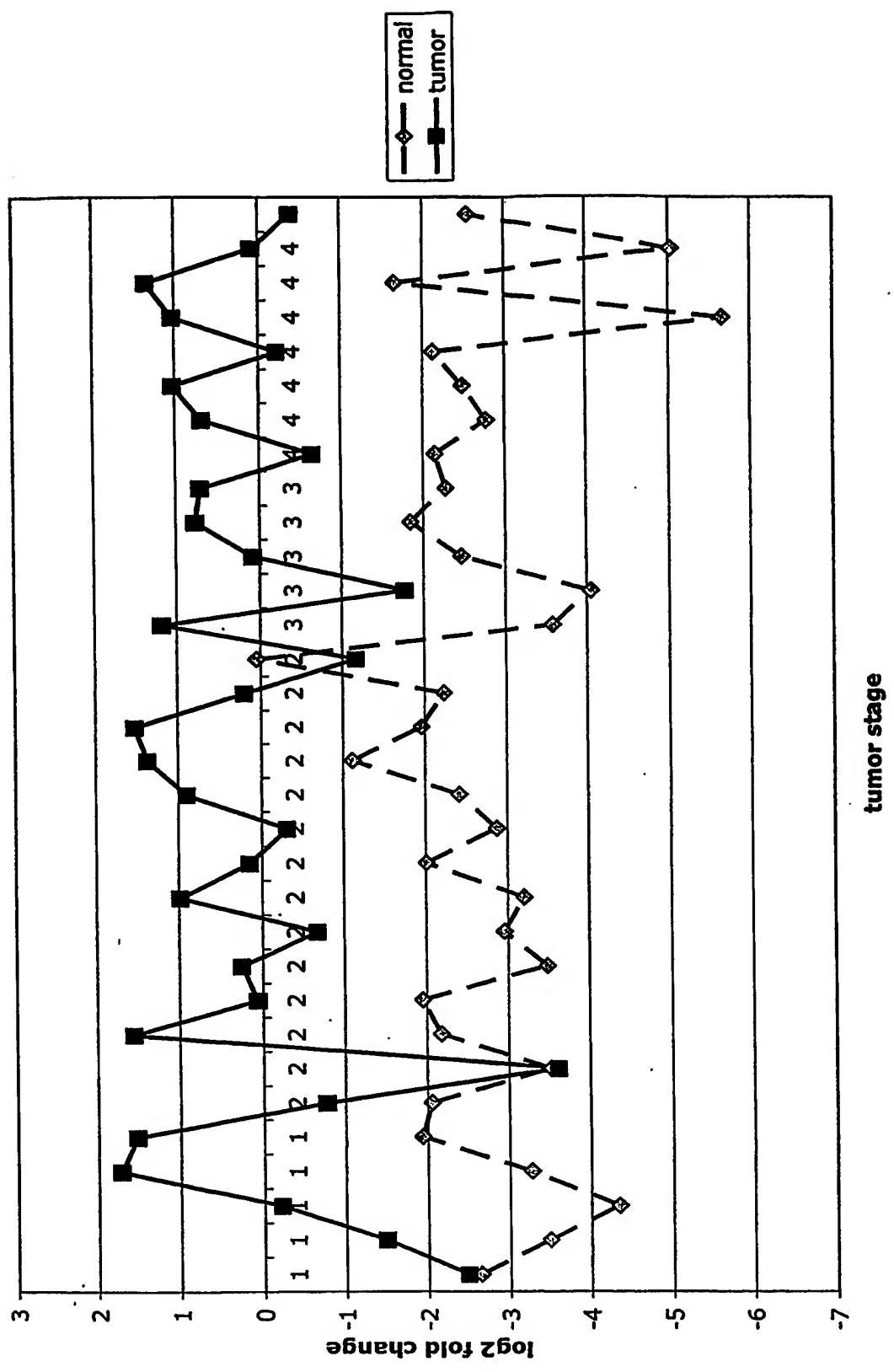


Fig. 10o ASAH1

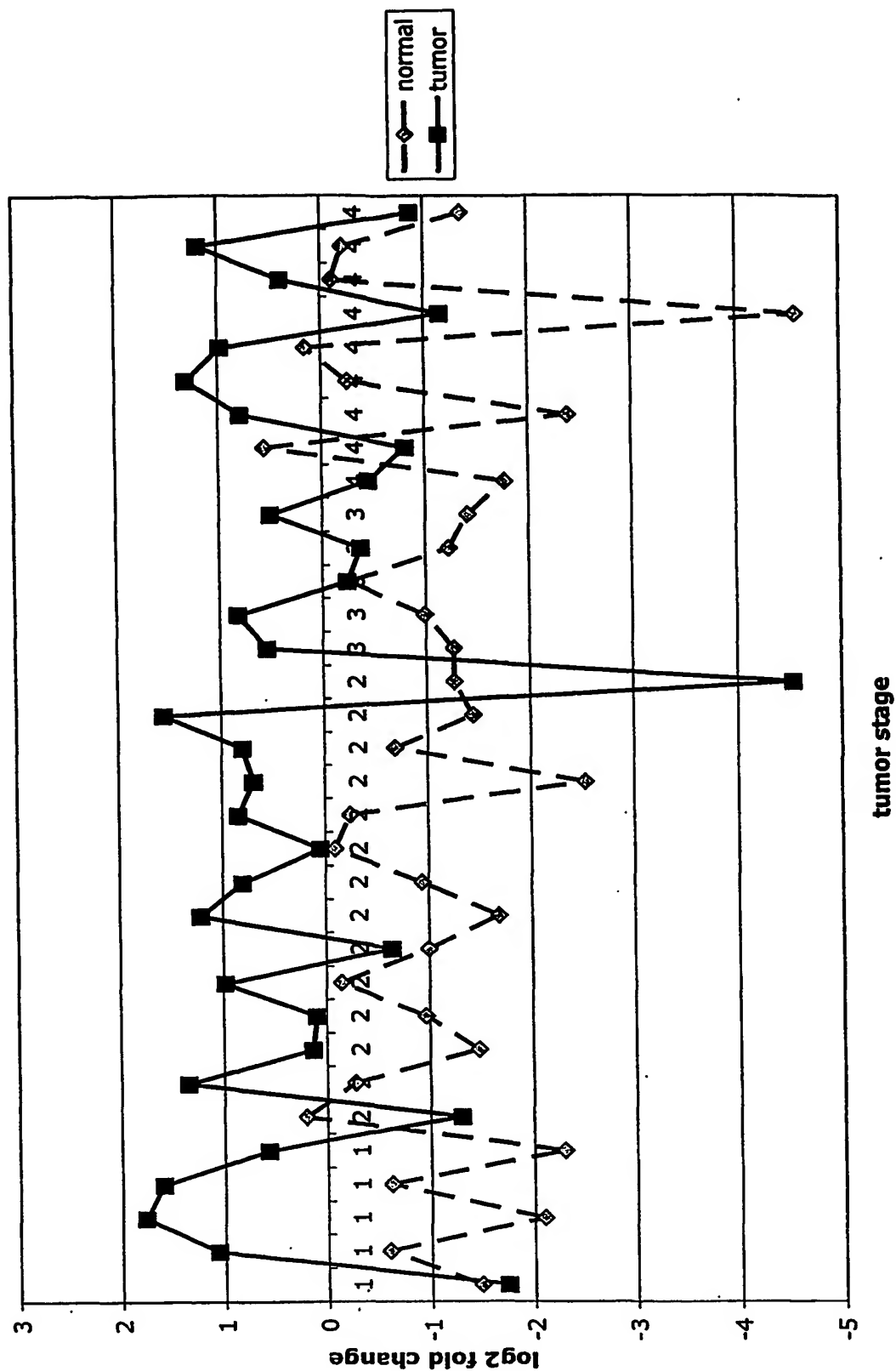


Fig. 10p SPP1

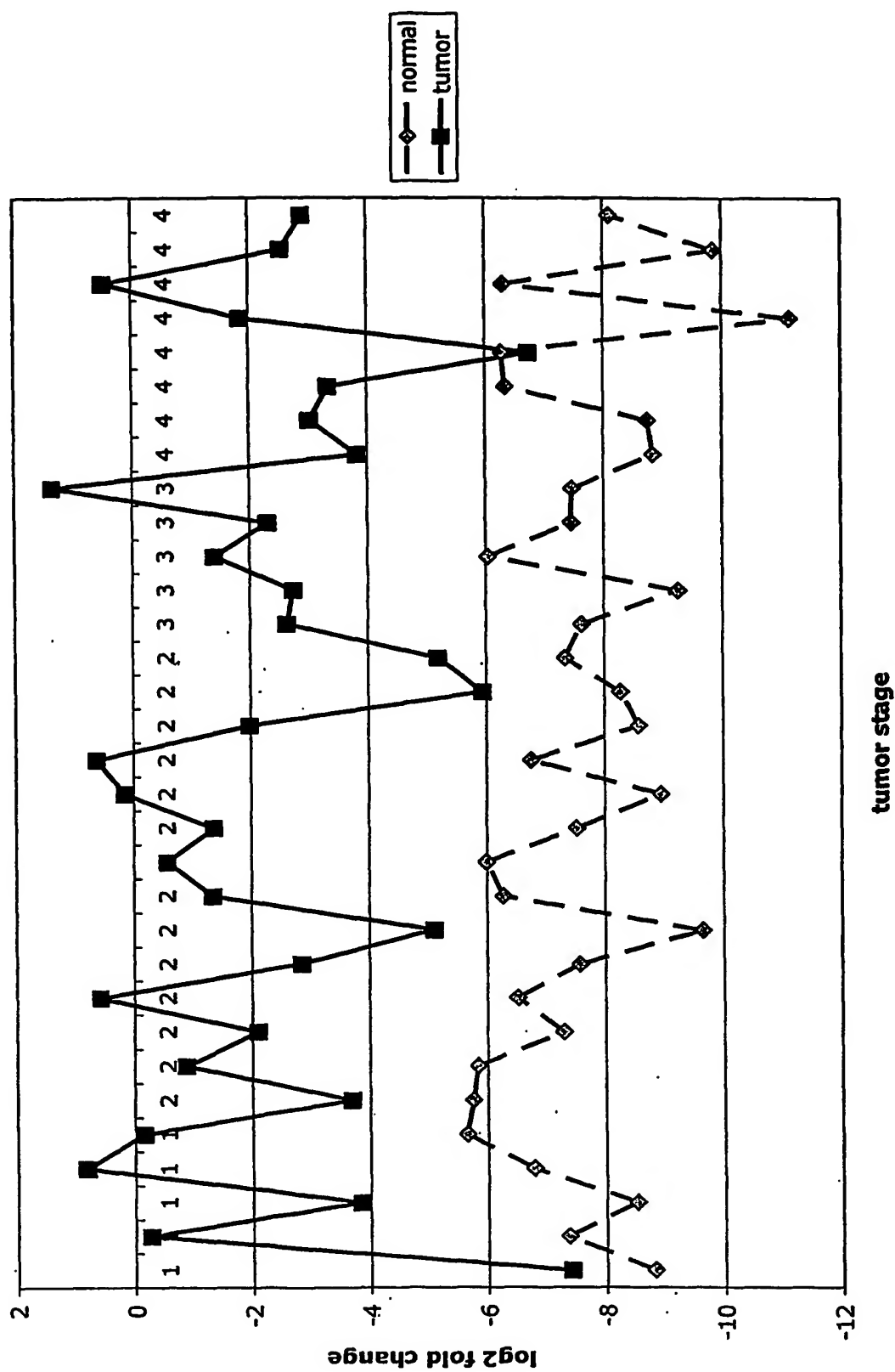


Fig. 10q SFRP2

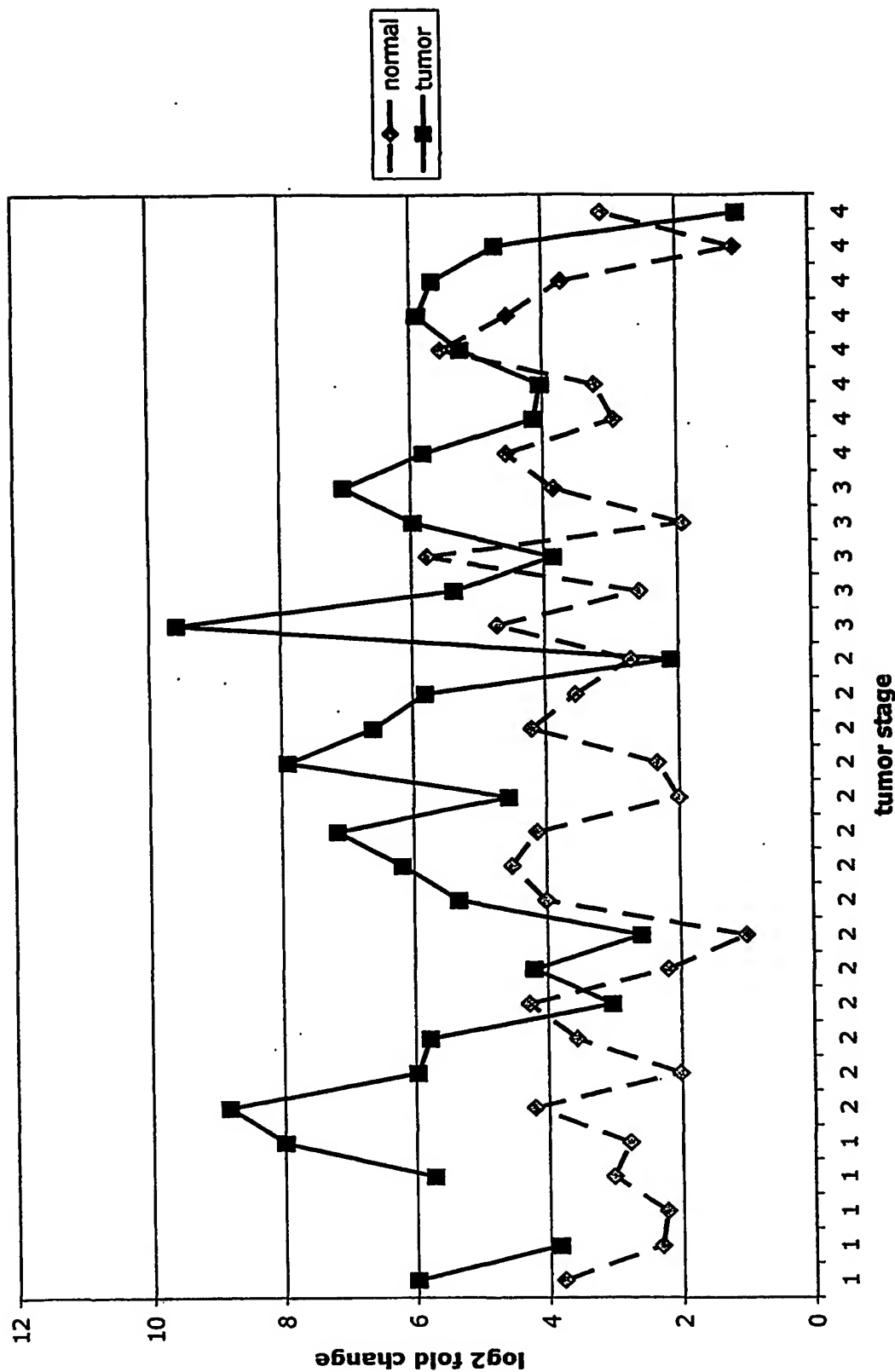


Fig. 10s SPARC

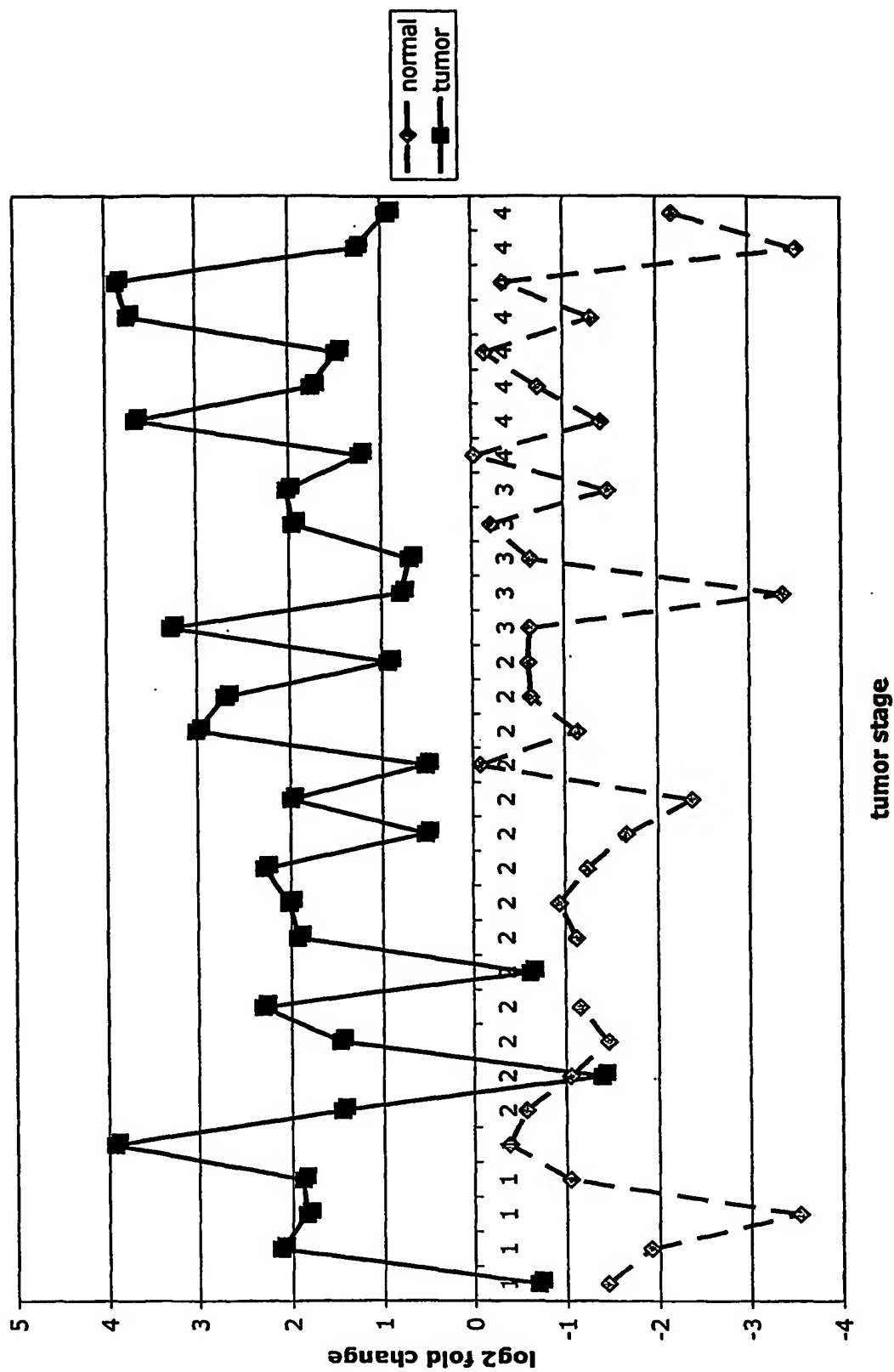


Fig. 10t PRSS11

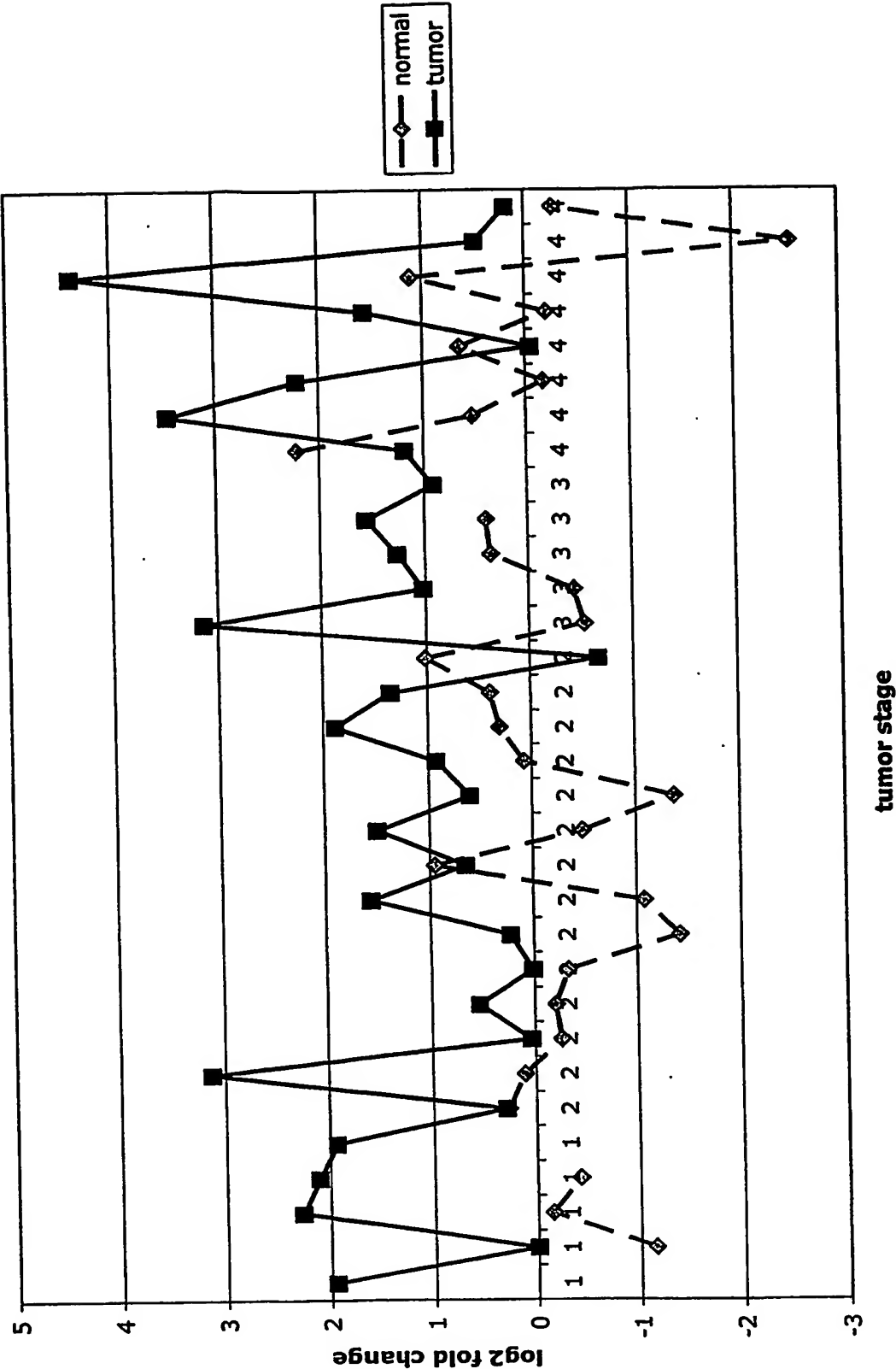
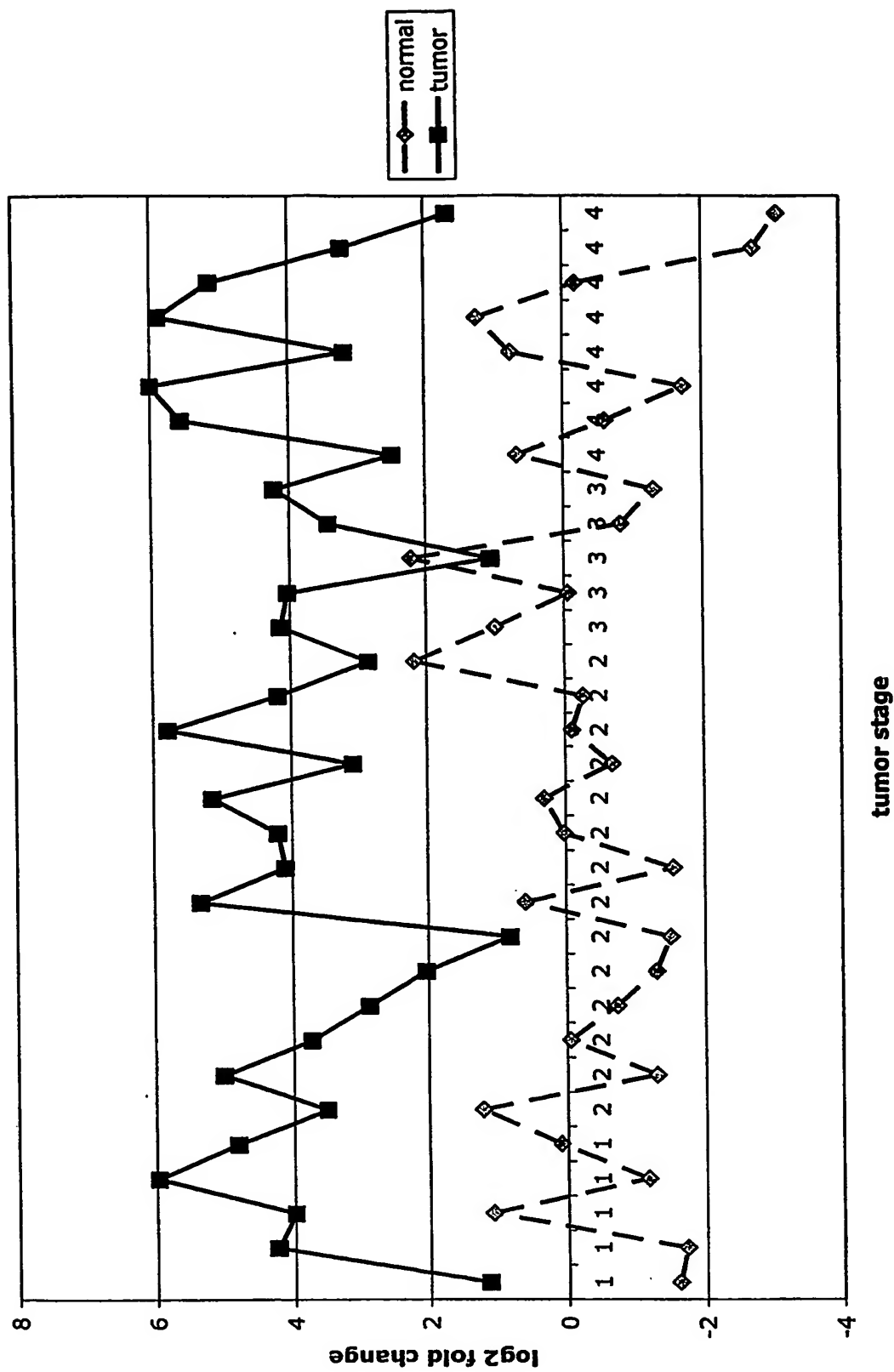


Fig. 10u THBS2



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Fig. 10v TG

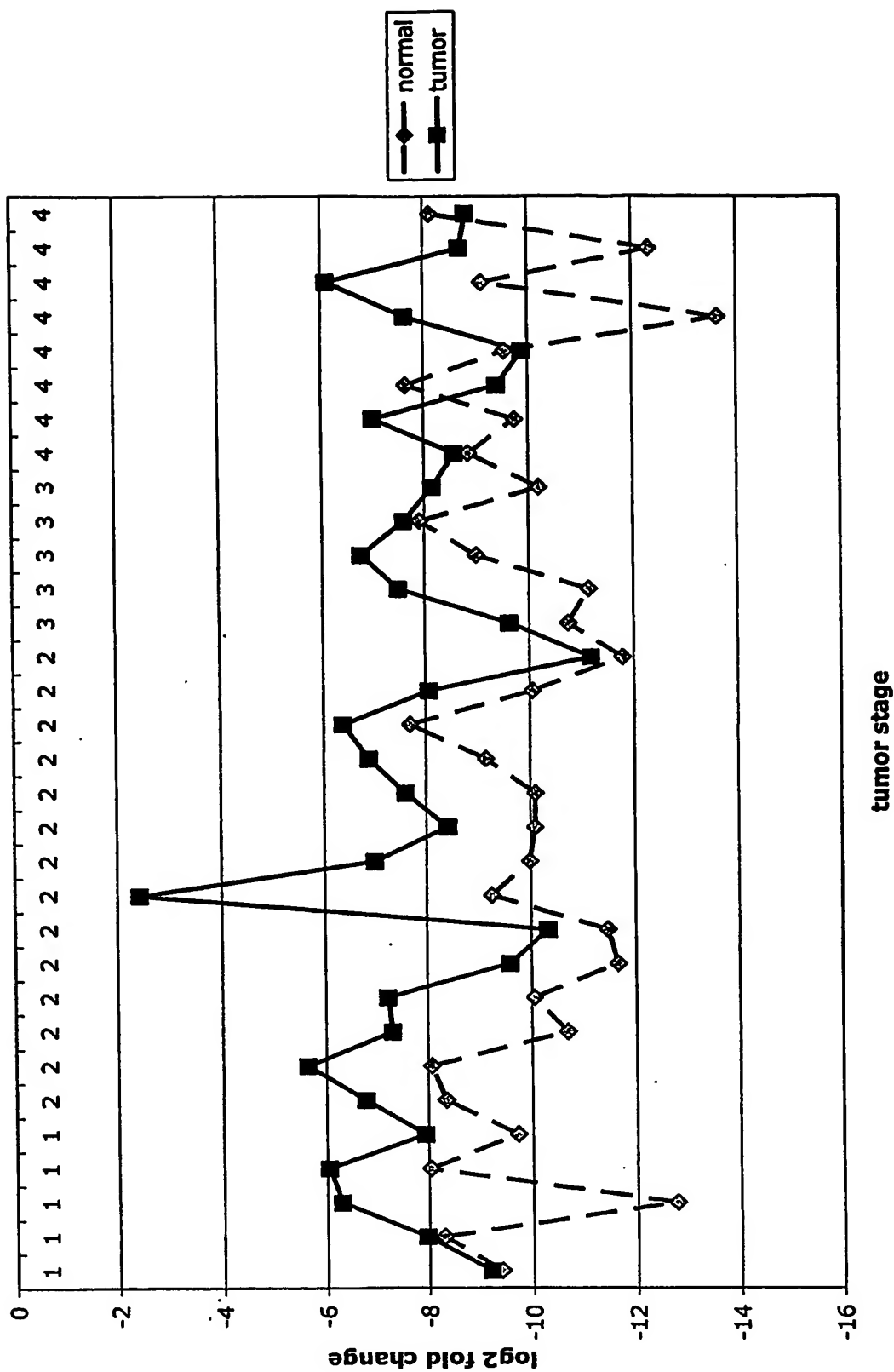


Fig. 10w TGFB1

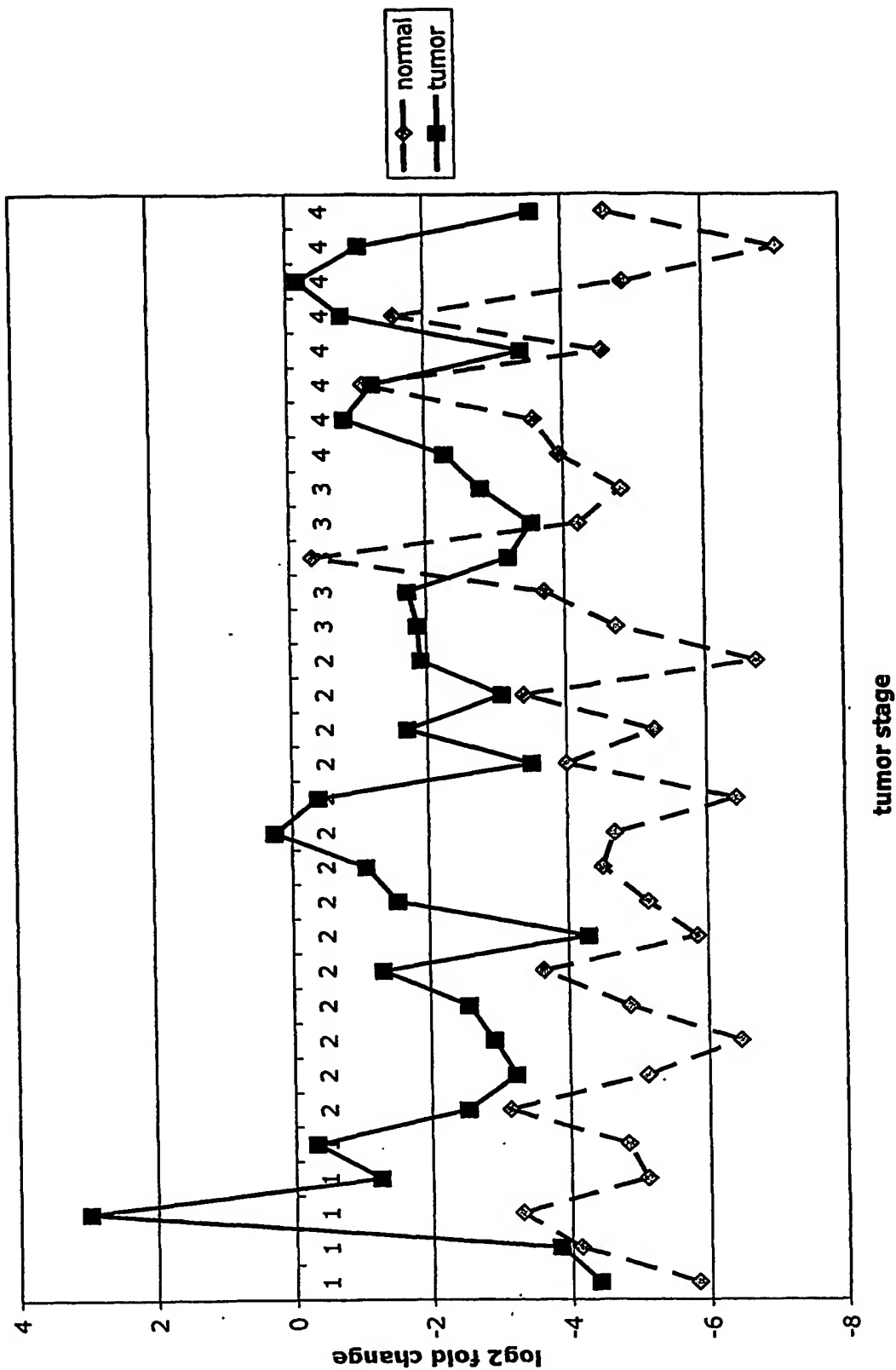


Fig. 10x CGR11

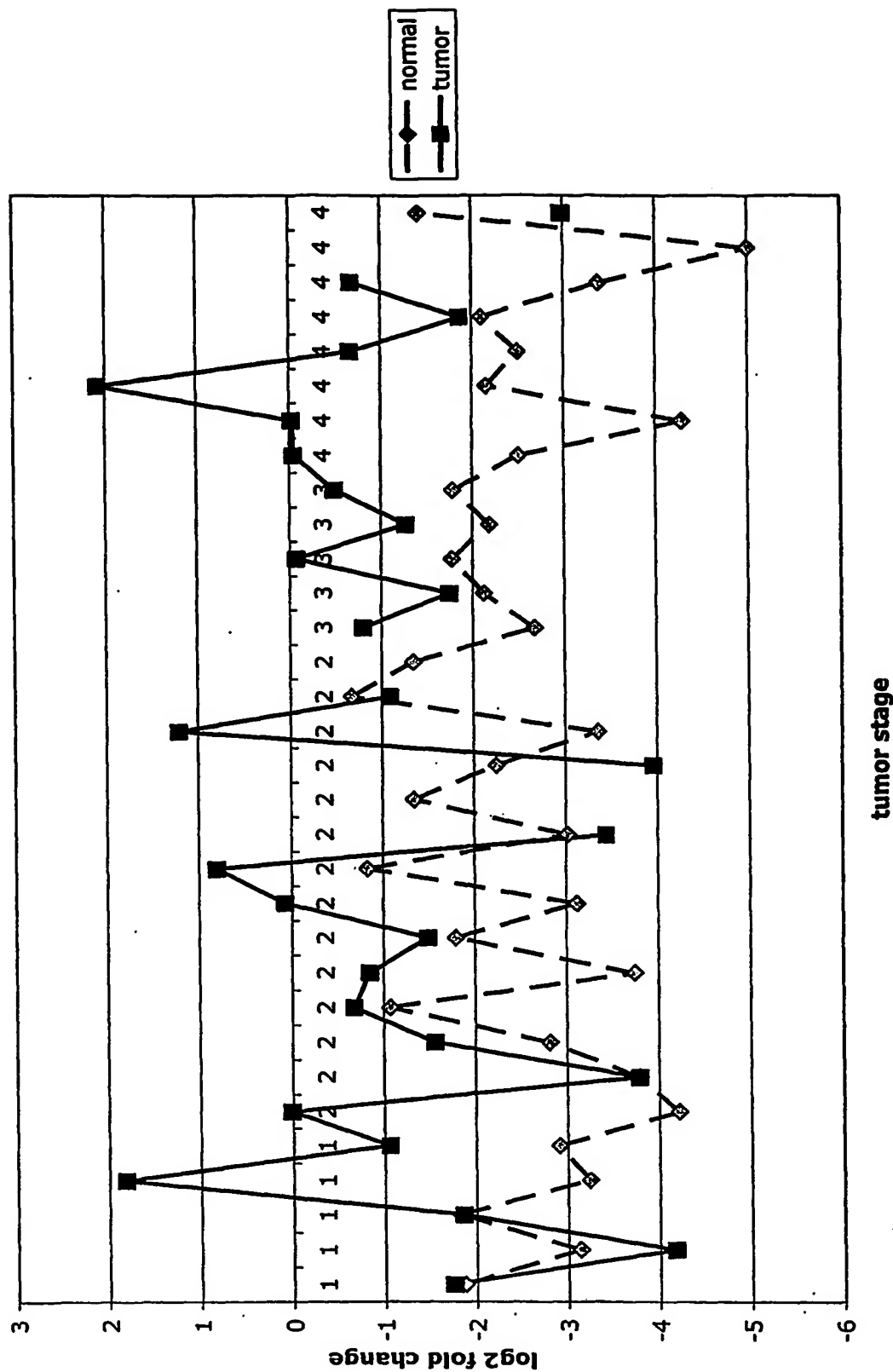


Fig. 10y SERPINH1

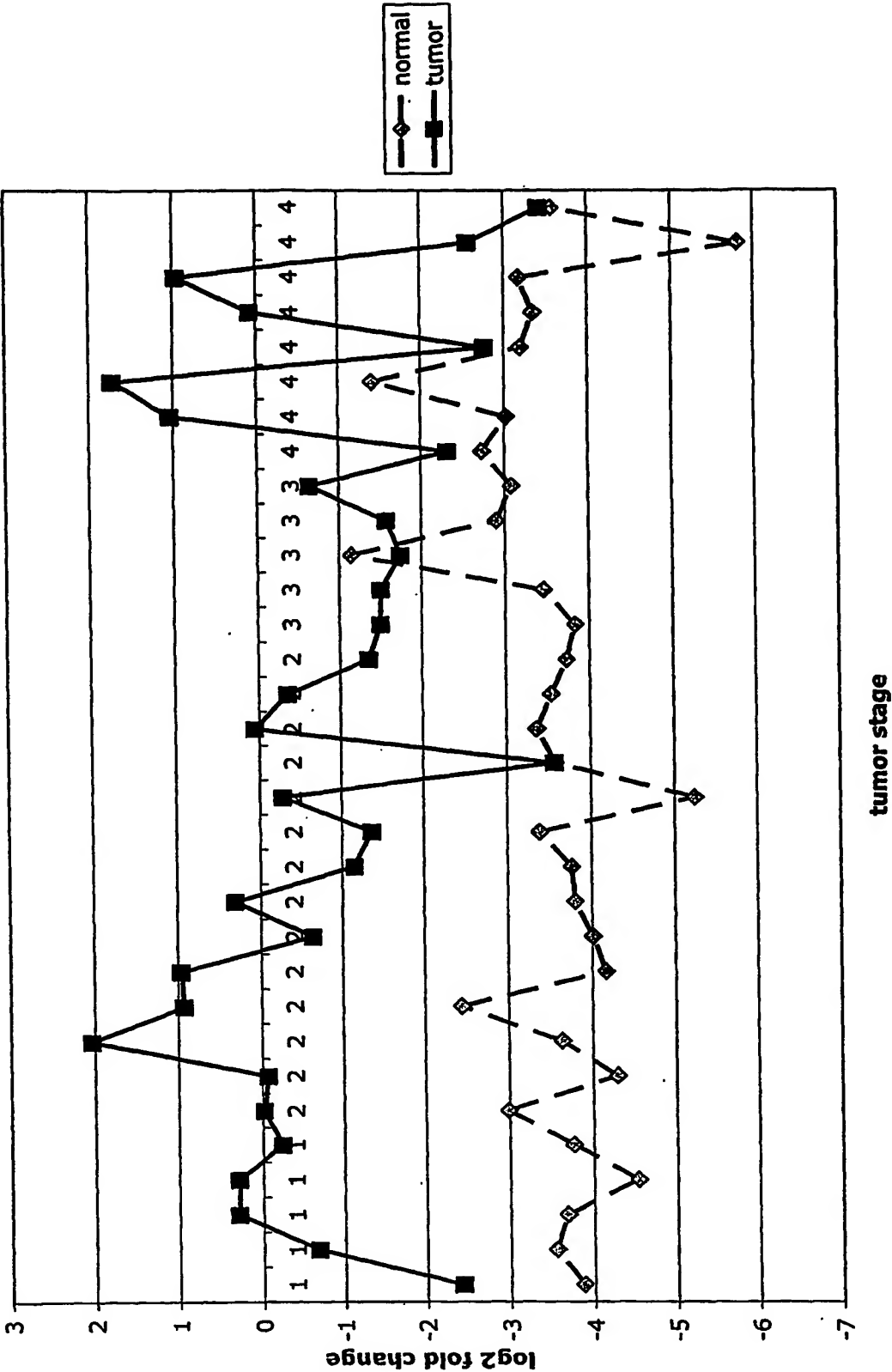


Fig. 10z MMP2

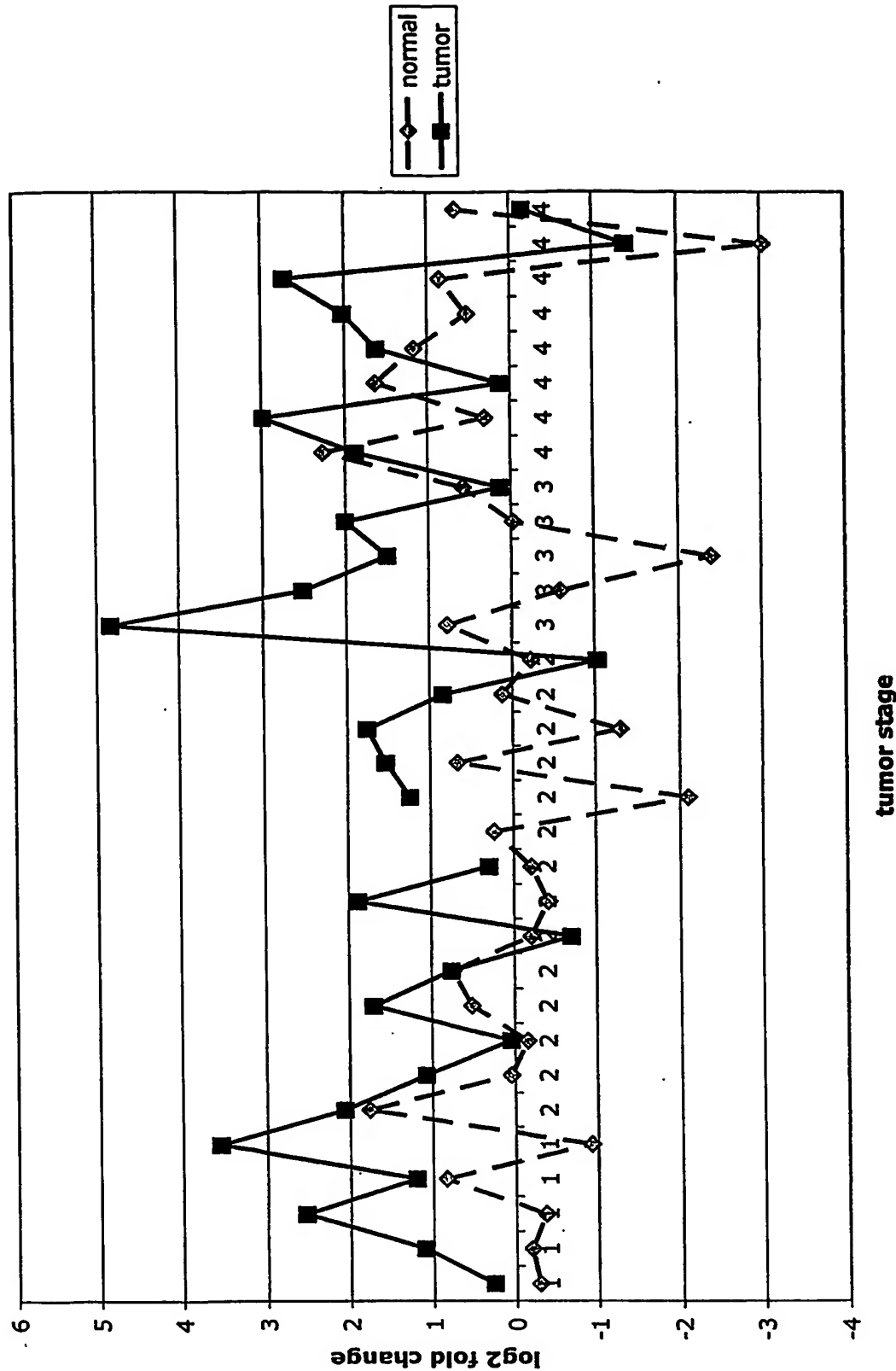


Fig. 10aa PCSK5

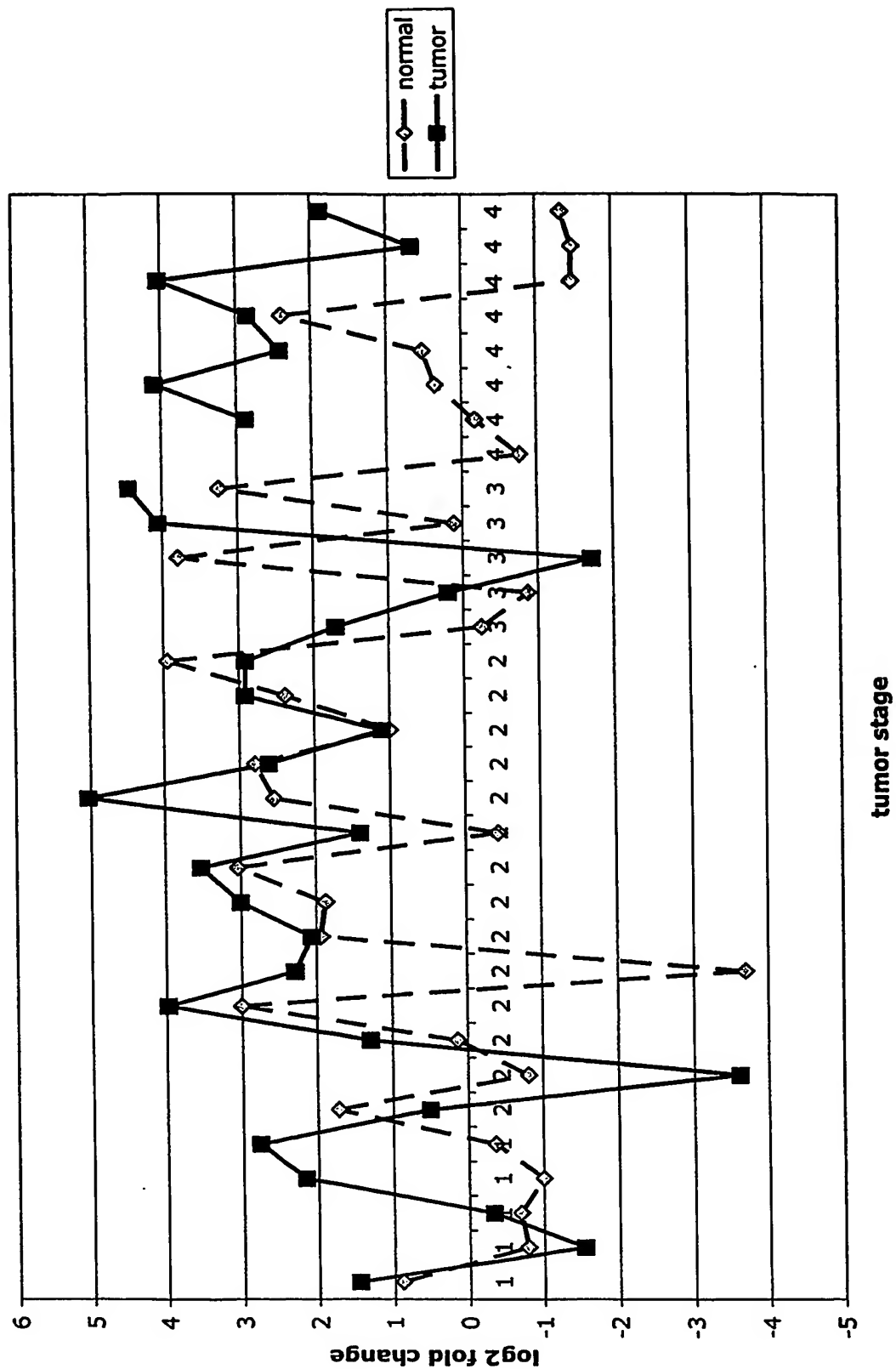


Fig. 10ab SERPINB5

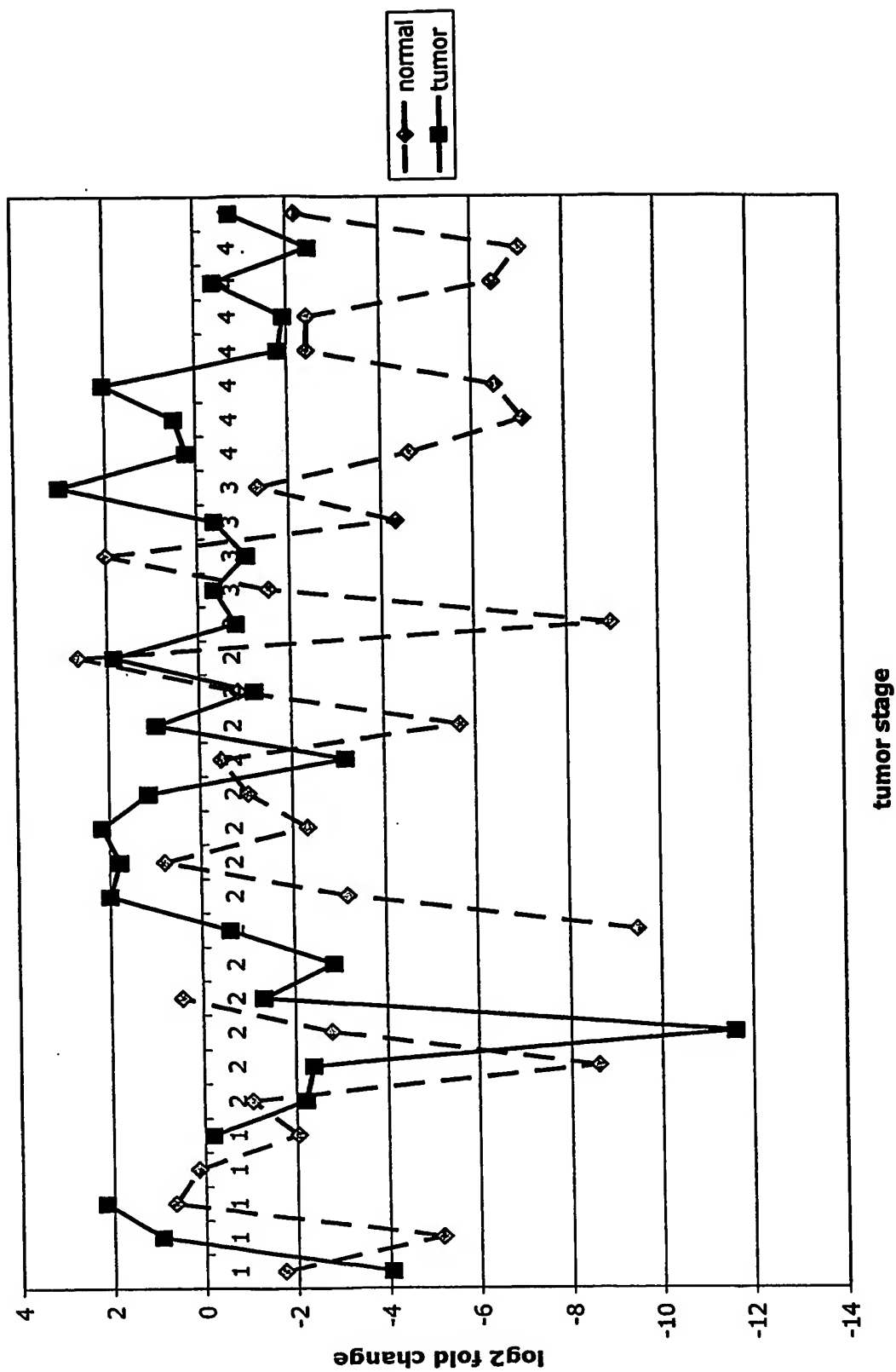


Fig. 10ac TGFb1

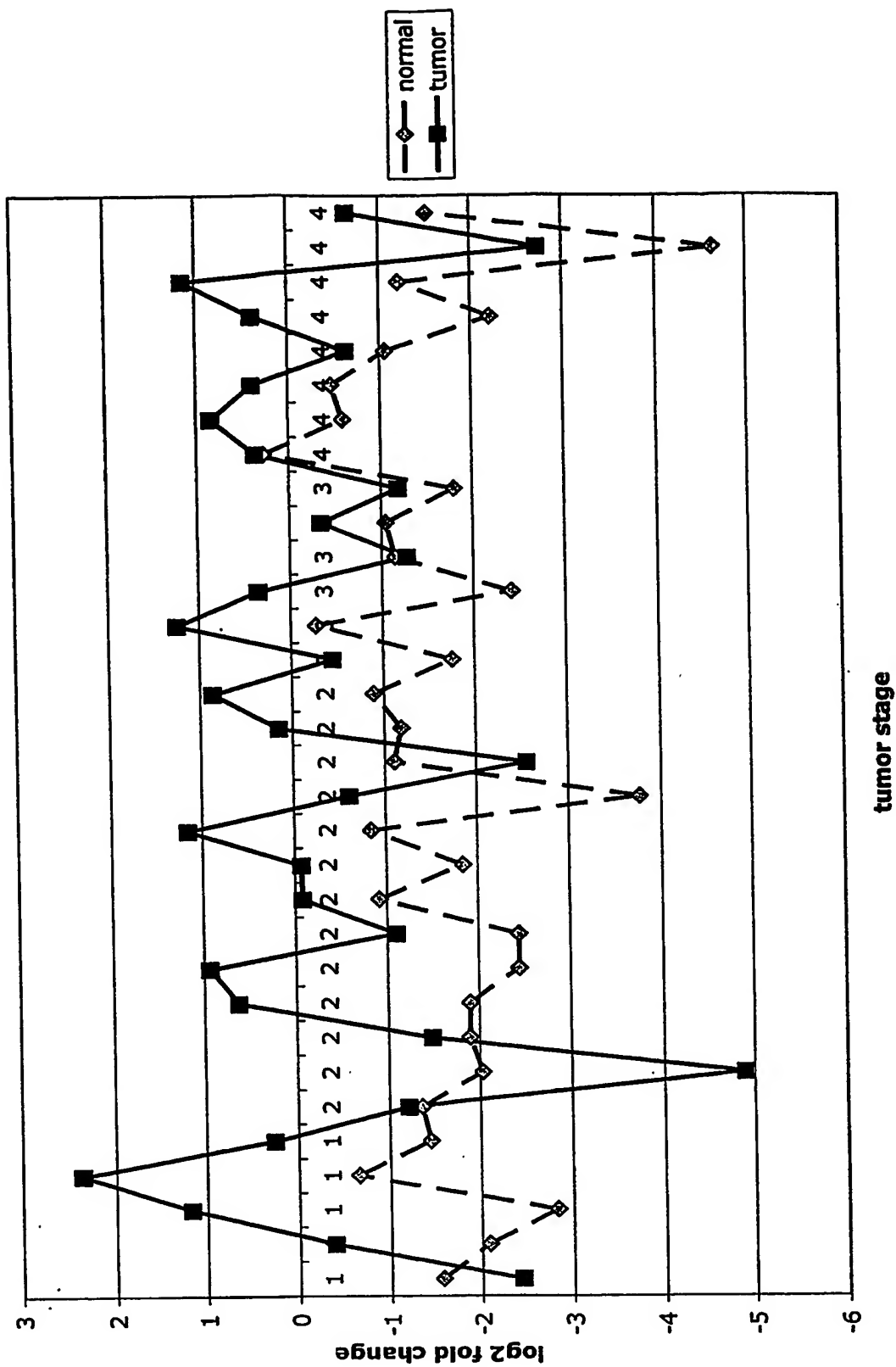


Fig. 10ad CEA

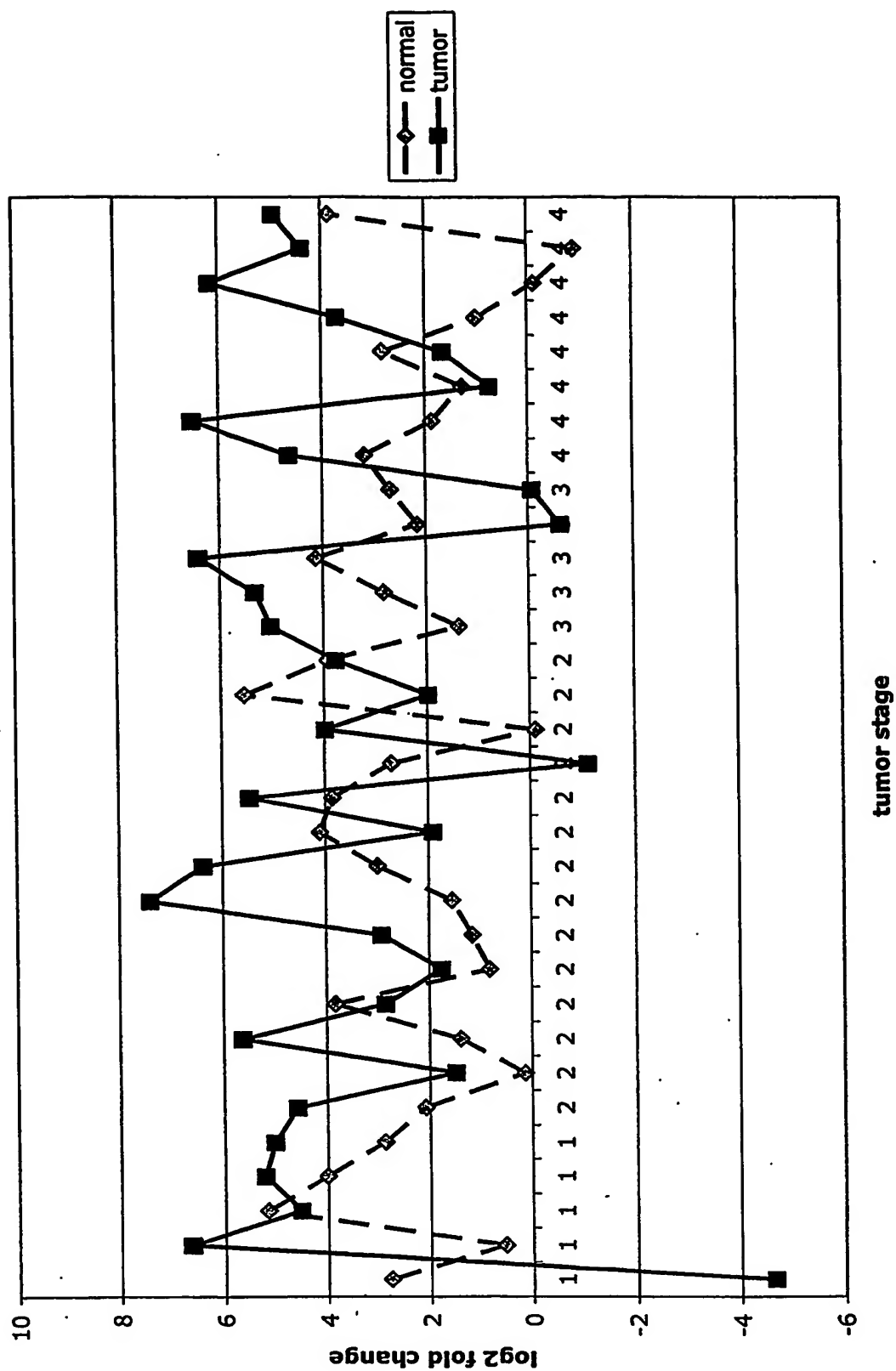


Fig. 11a Adlican

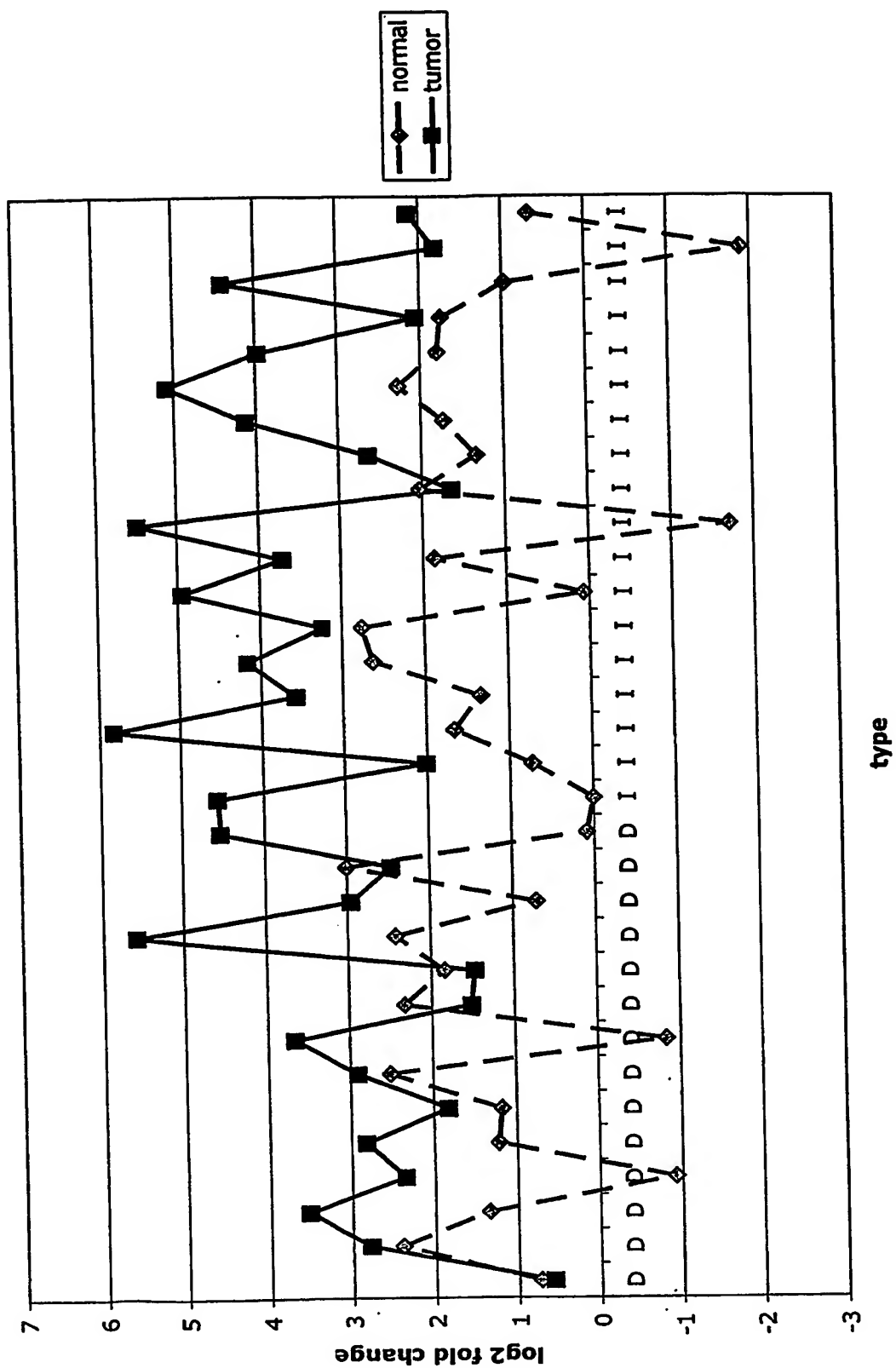


Fig. 11b ASPN

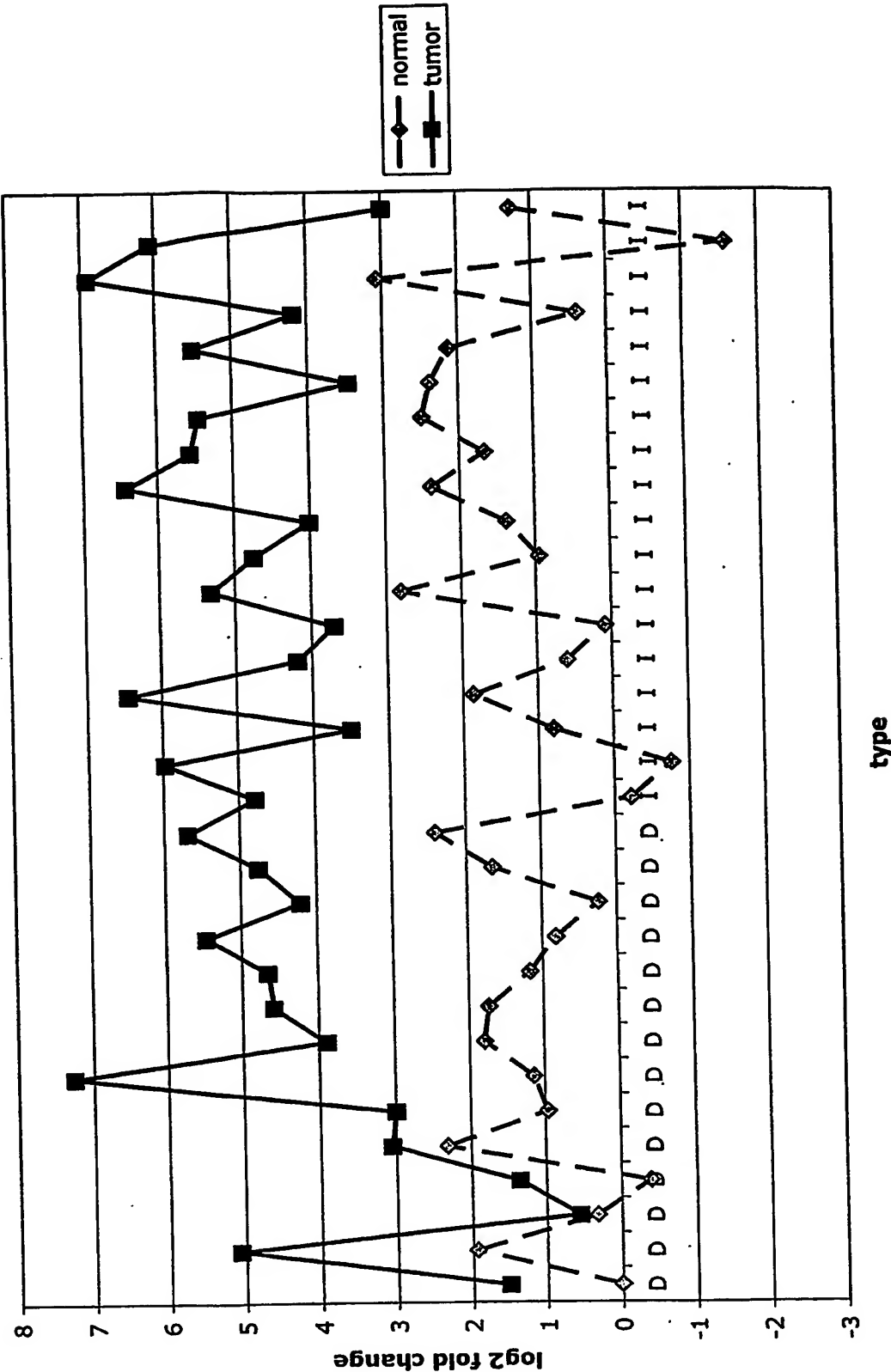
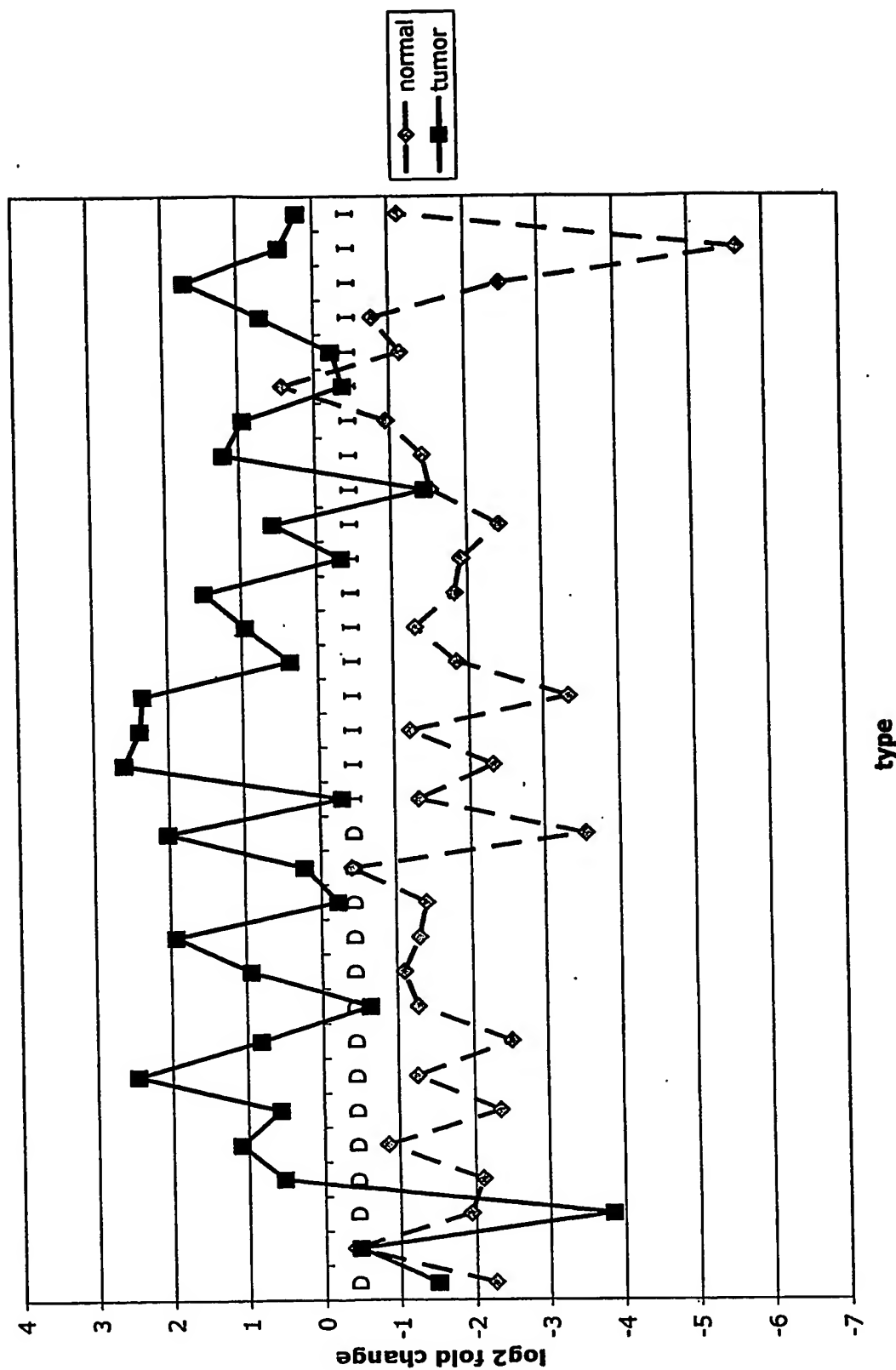


Fig. 11c CSPG2



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Fig. 11d CST1,2,4

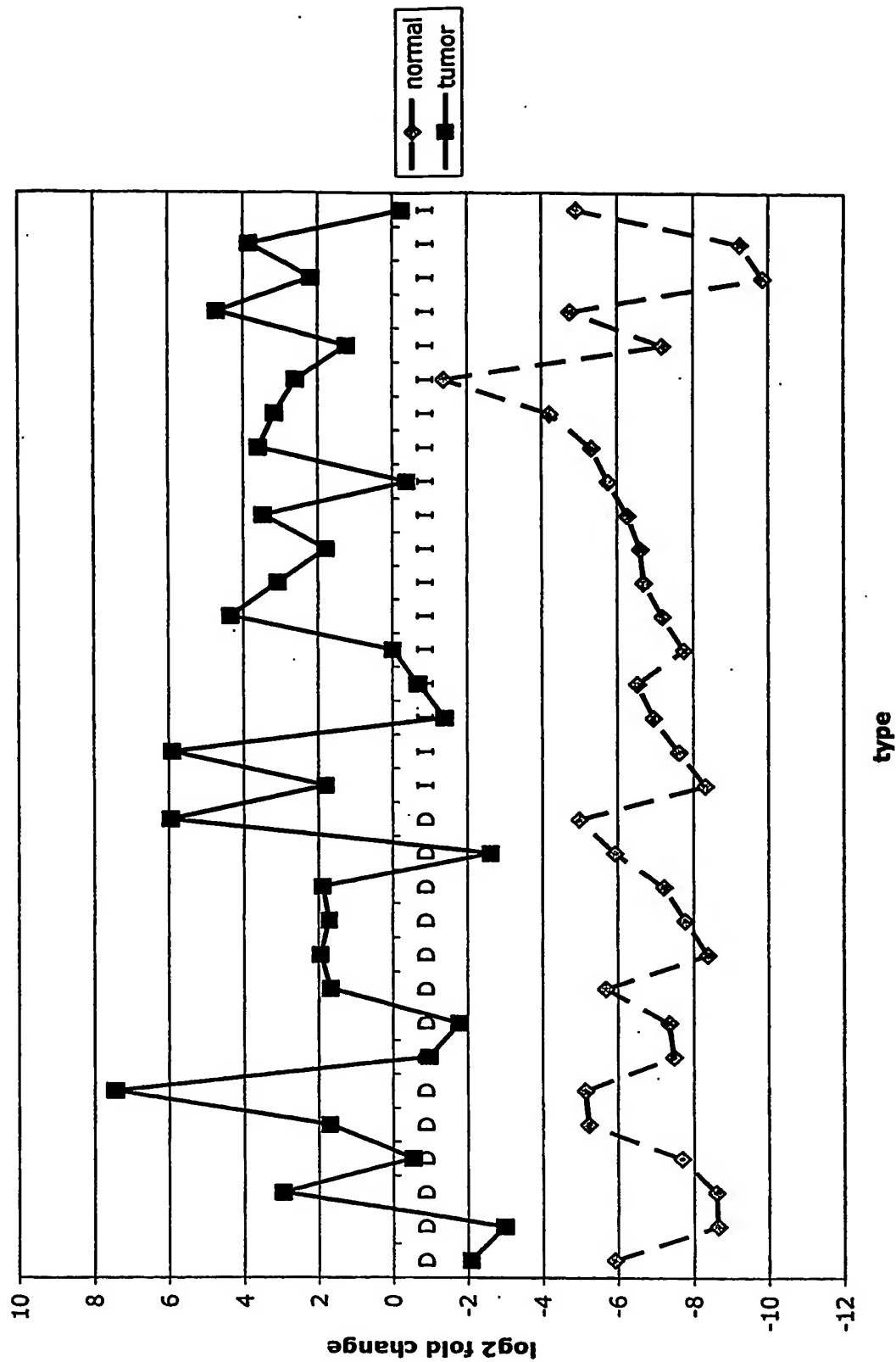


Fig. 11e EFEMP2

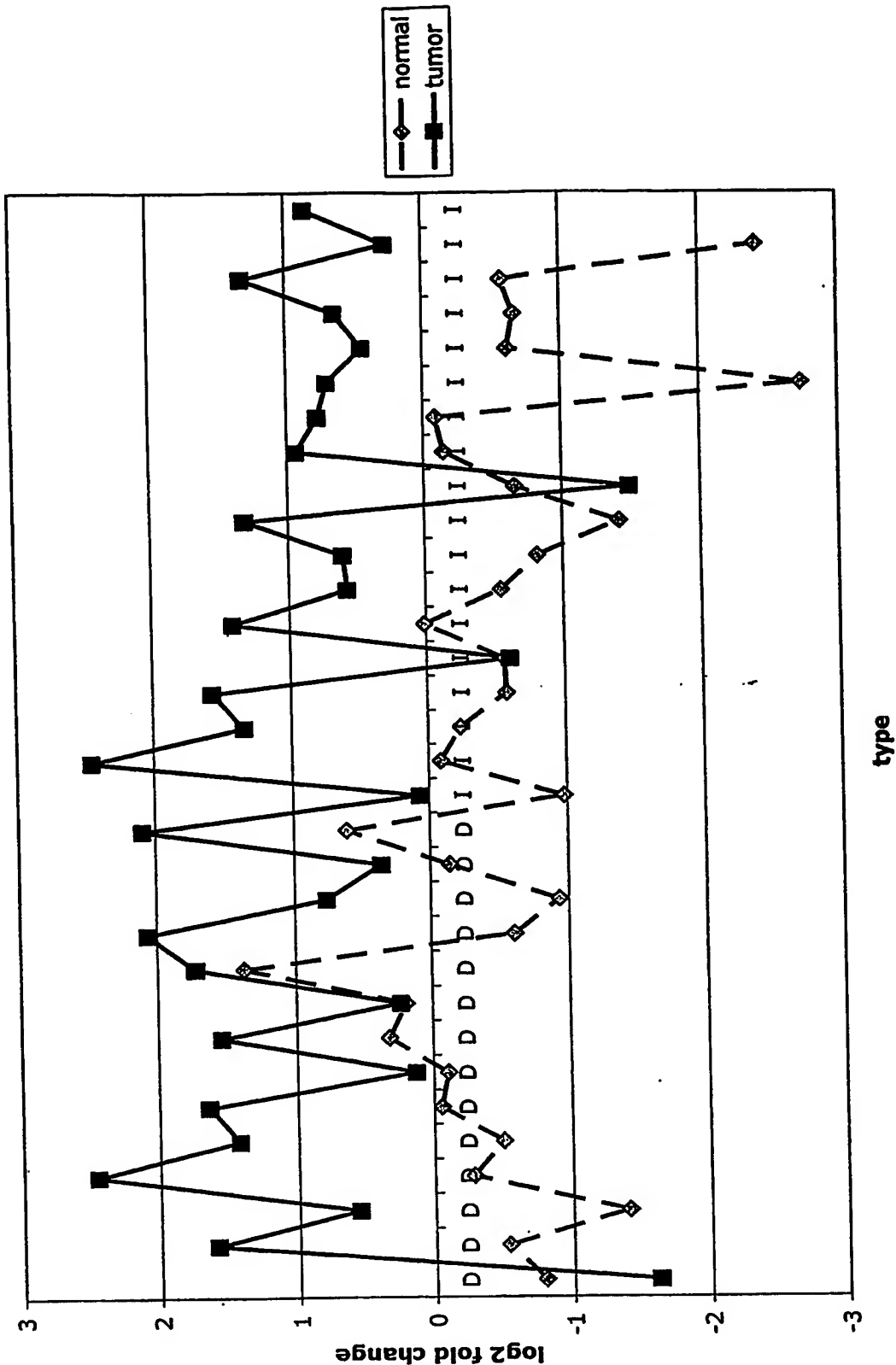


Fig. 11f GGH

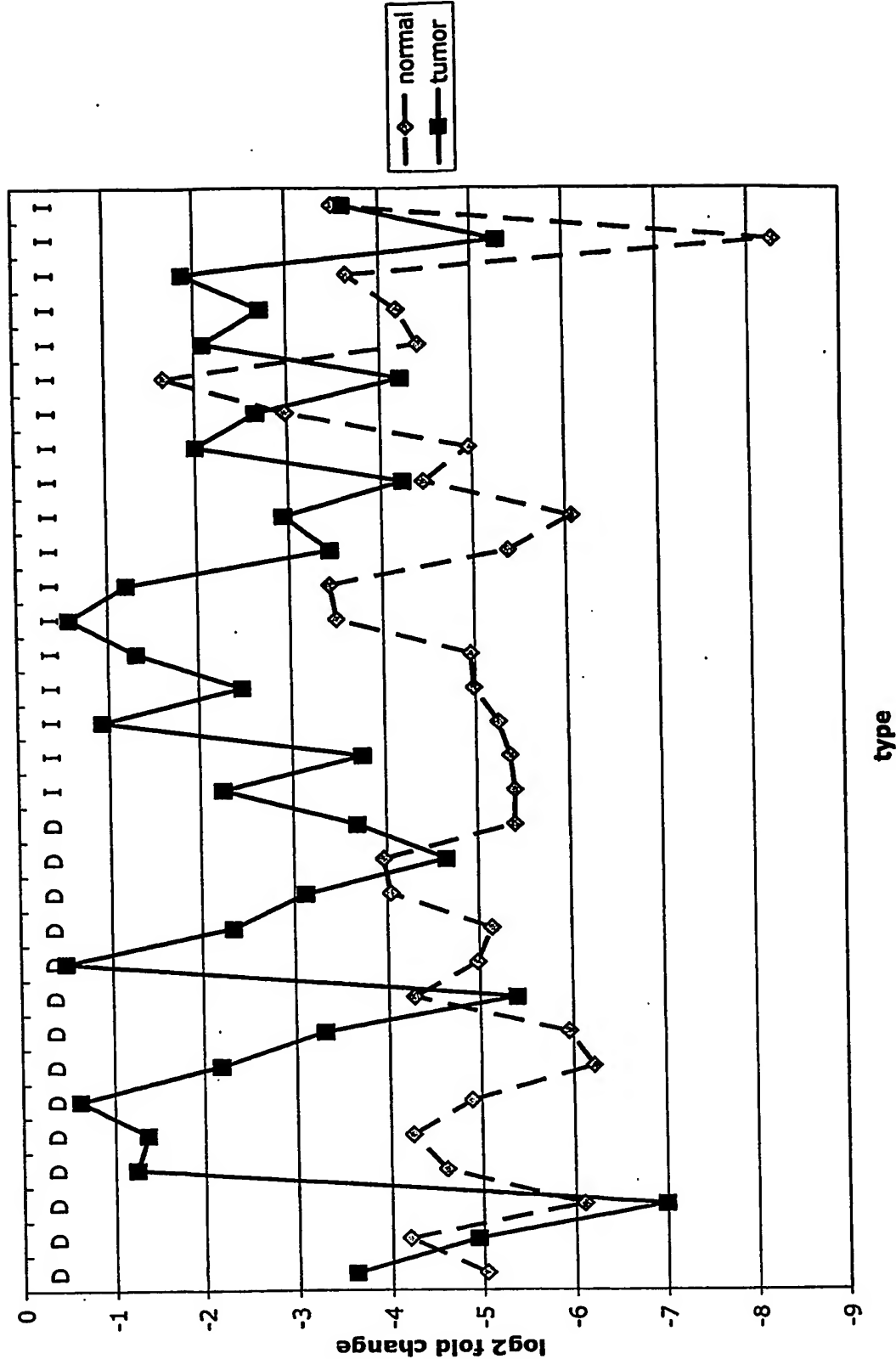


Fig. 11g INHBA

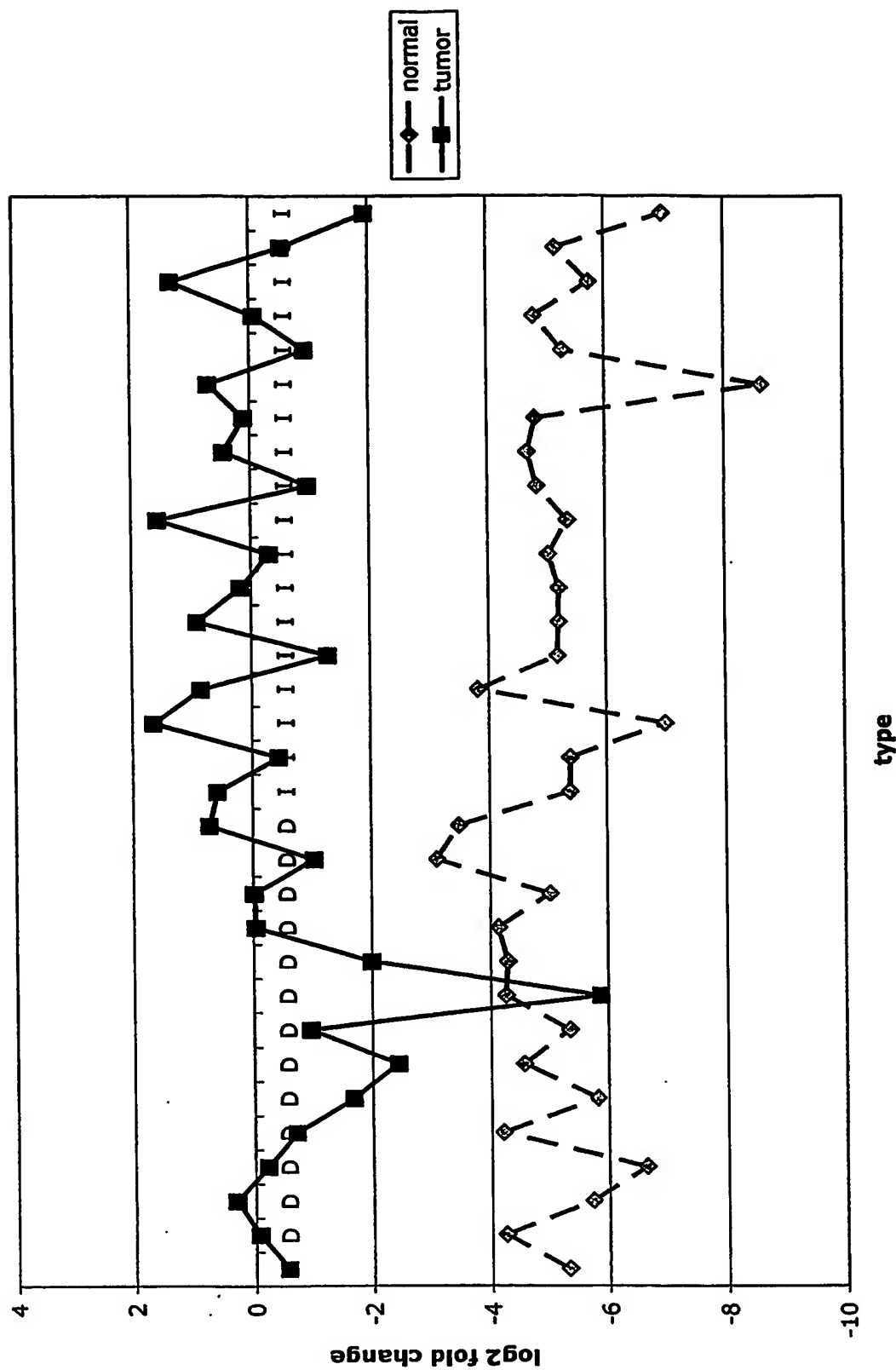


Fig. 11h IGFBP7

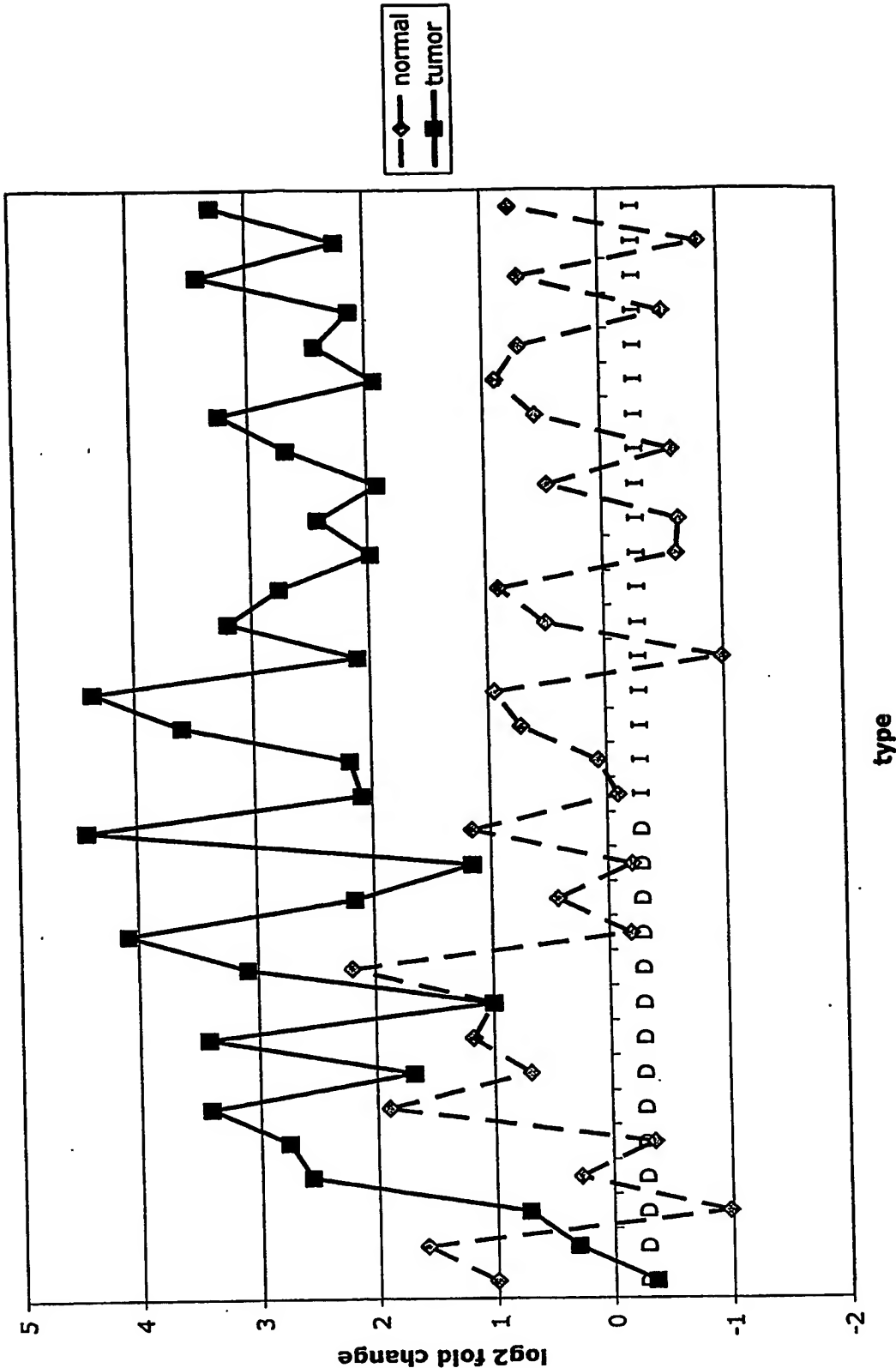


Fig. 11i KLK10

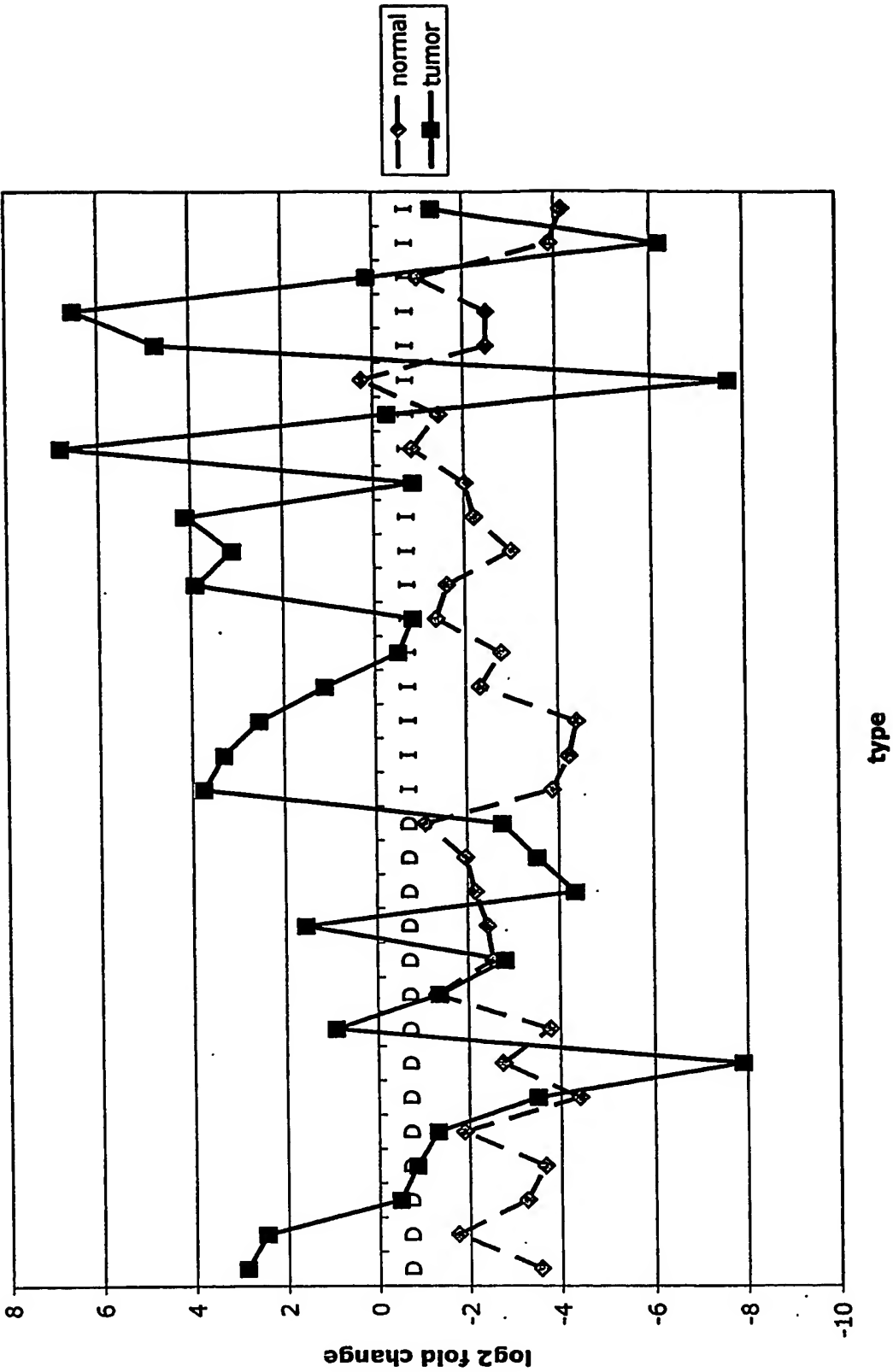
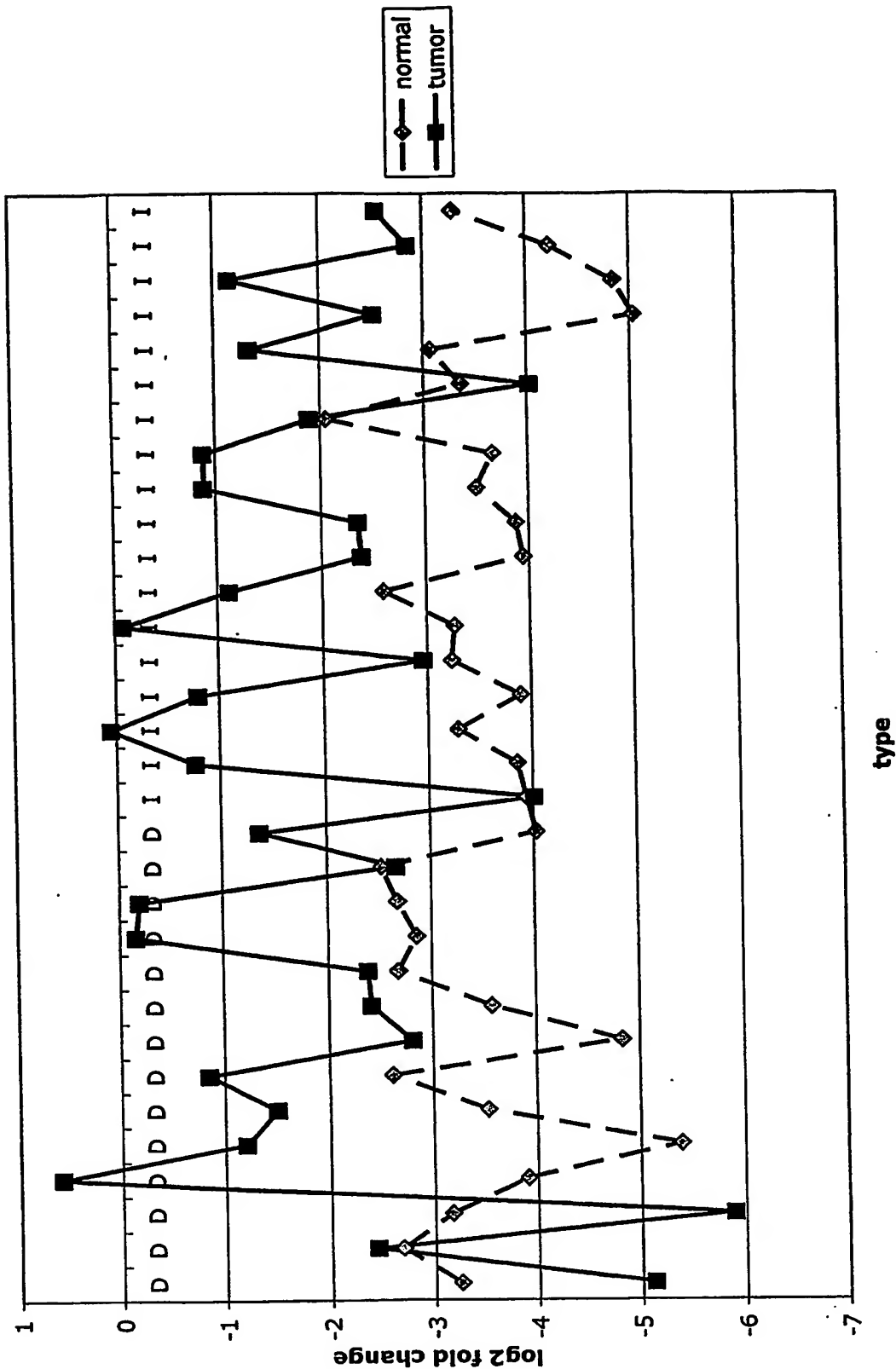


Fig. 11j LEPRE1



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Fig. 11k LUM

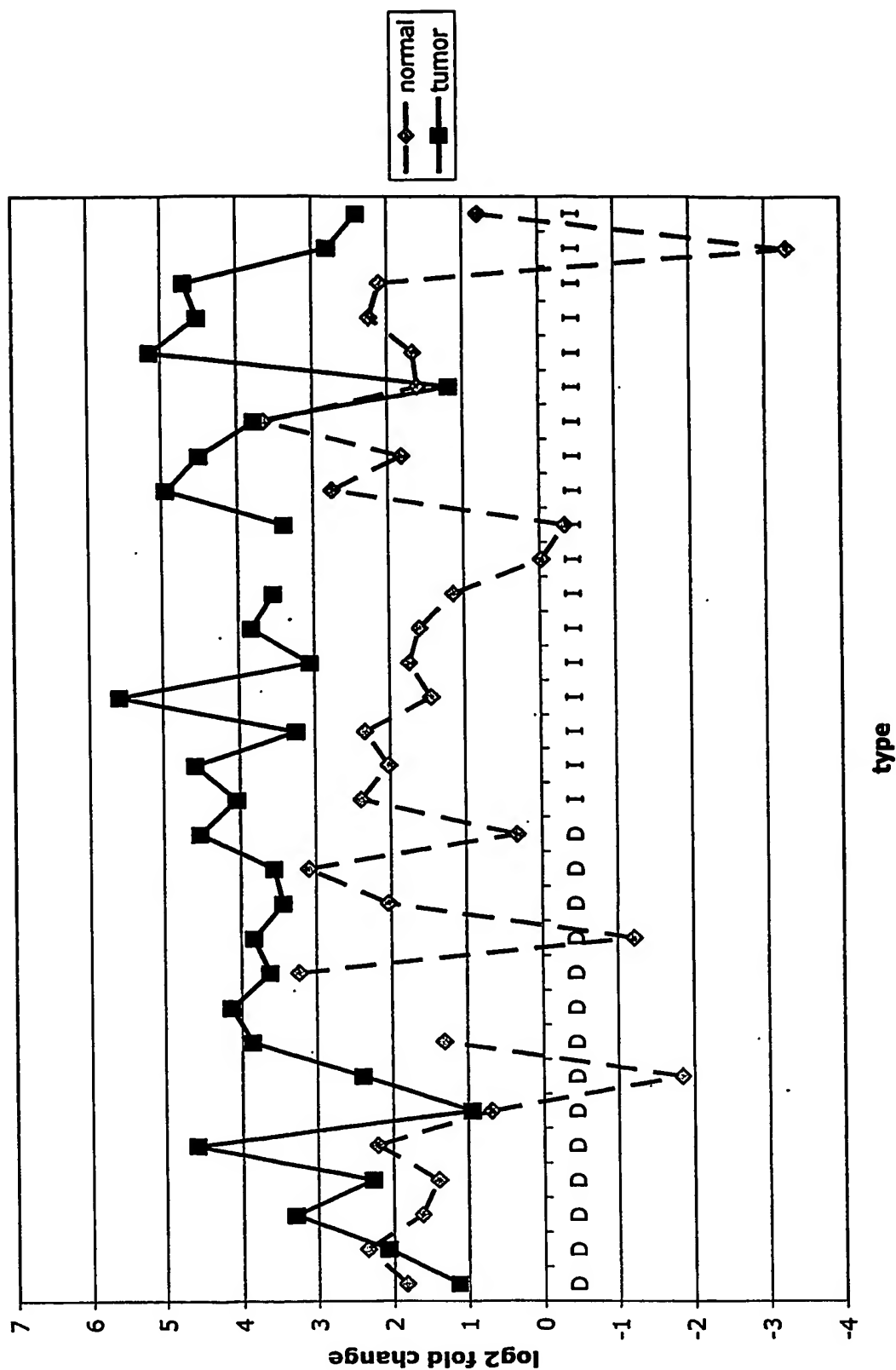


Fig. 11 LOXL2

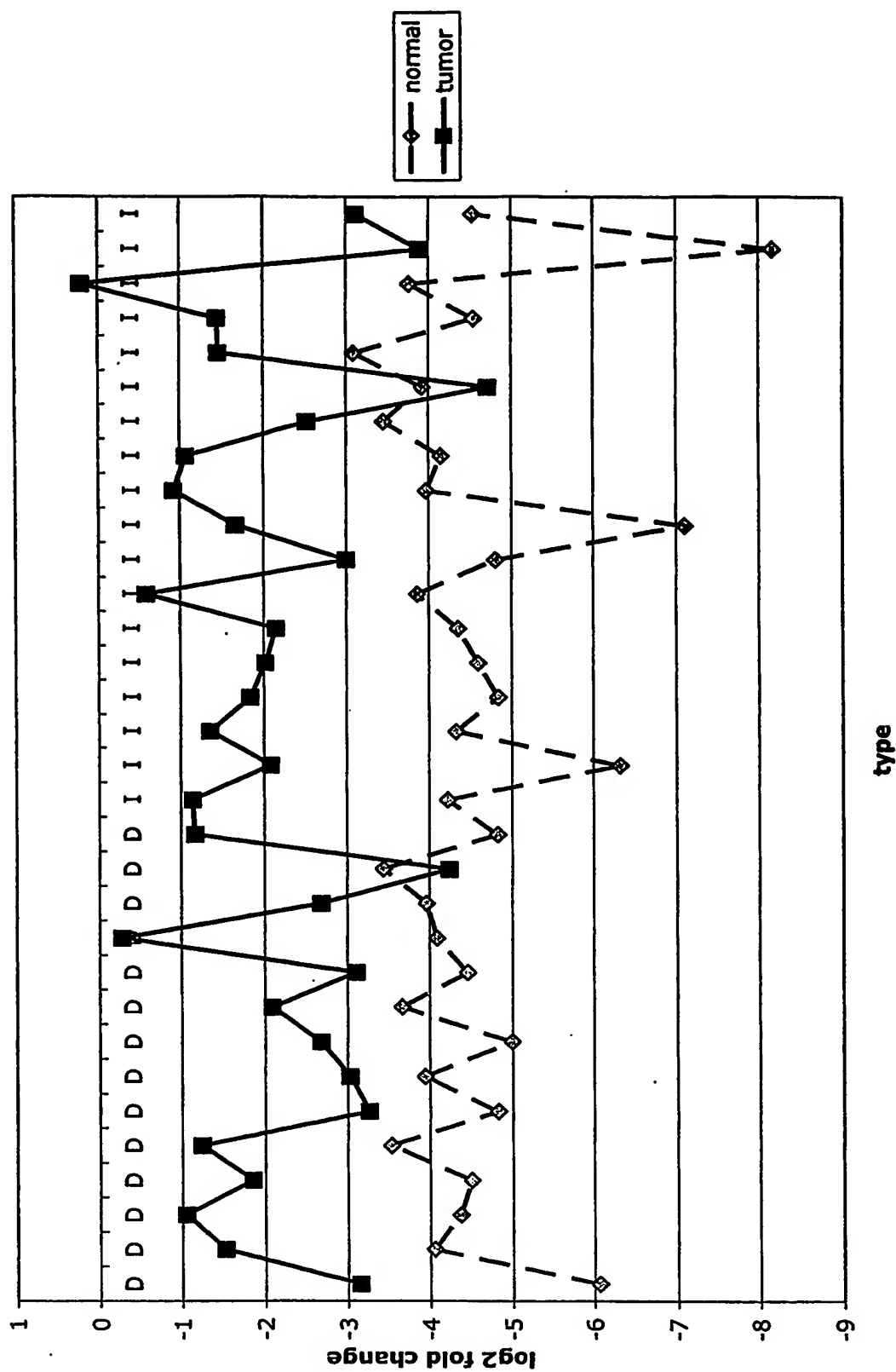


Fig. 11m MMP12

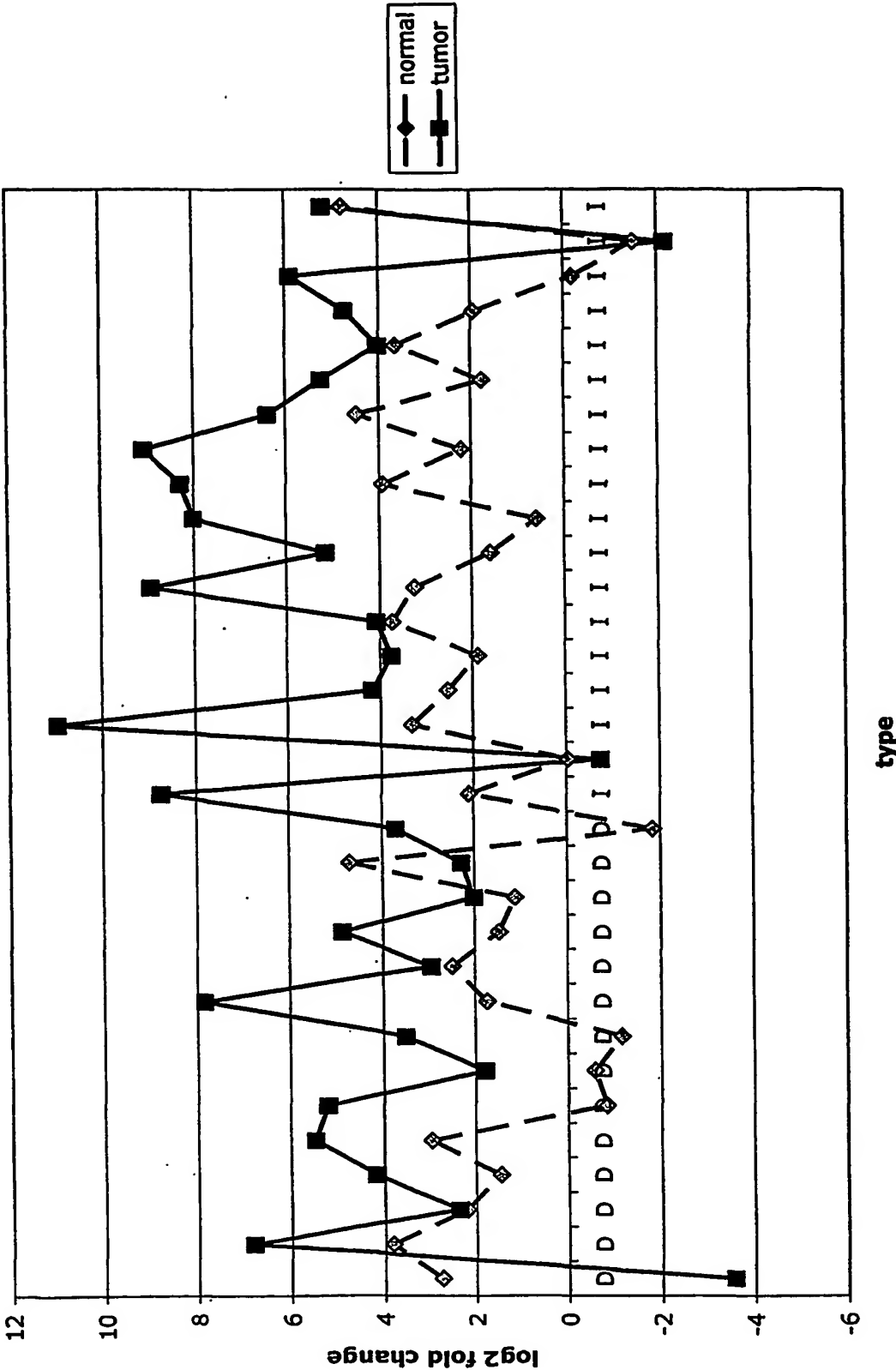


Fig. 11n TIMP1

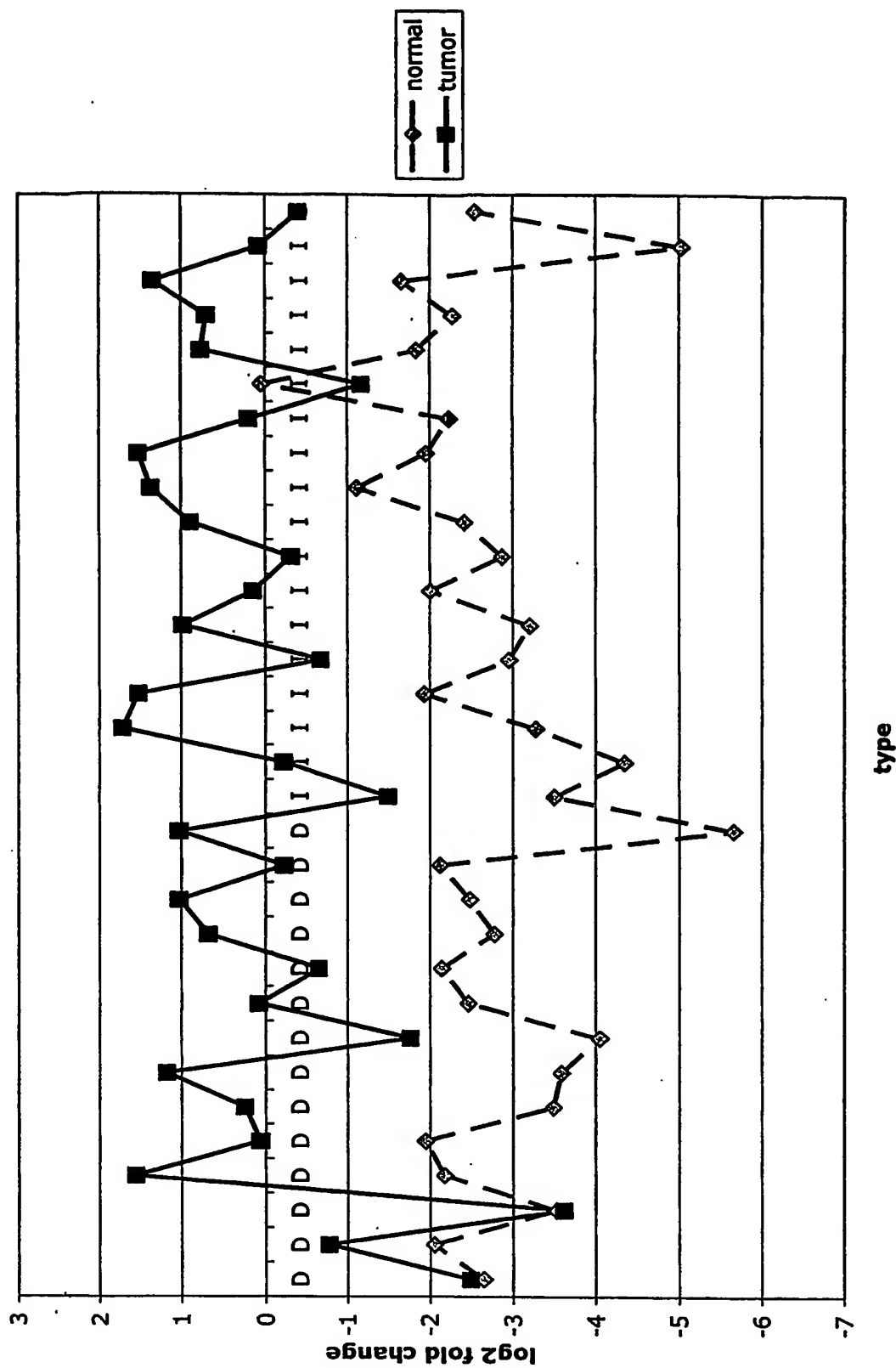


Fig. 11o ASAH1

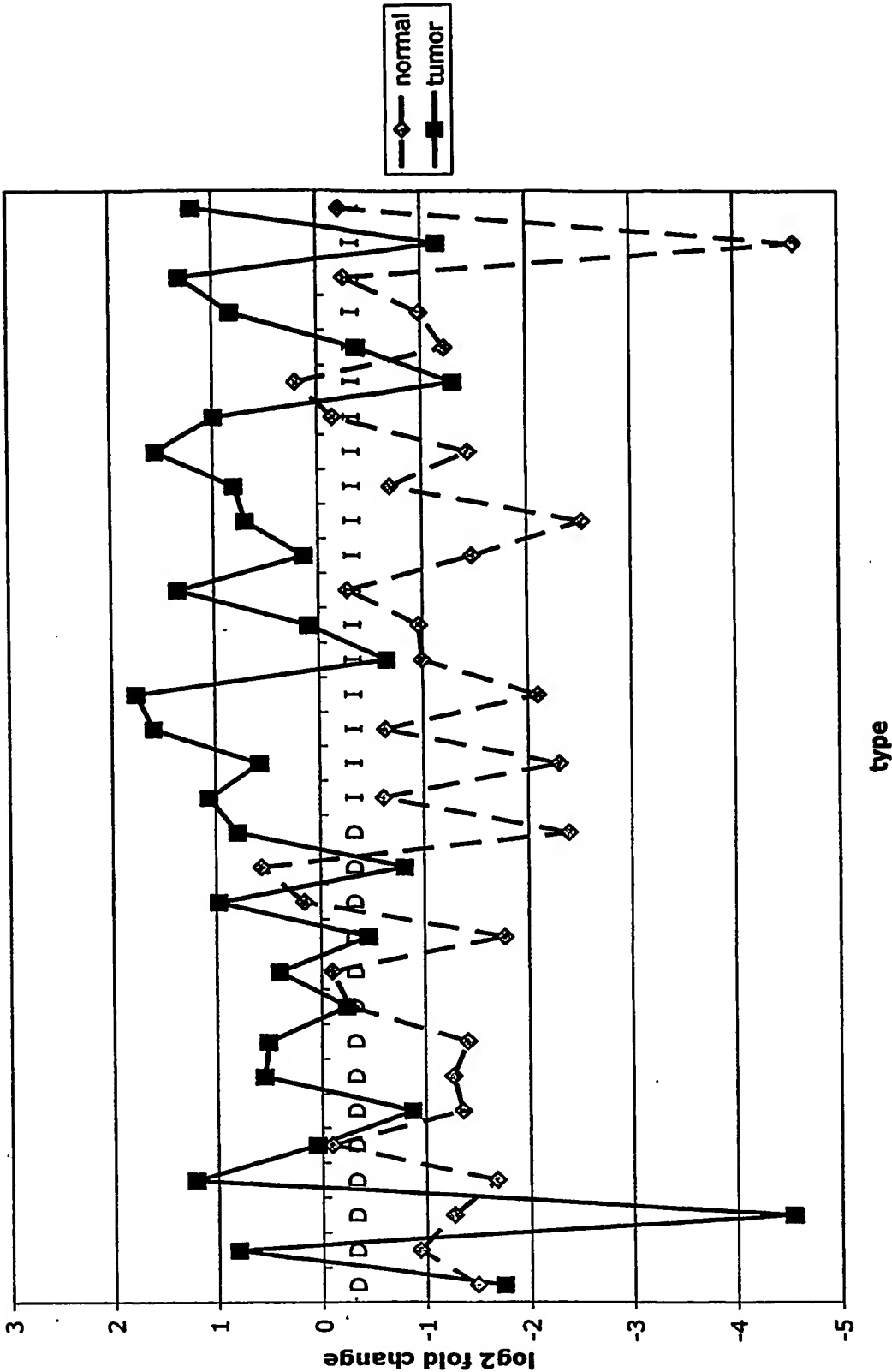


Fig. 11p SPP1

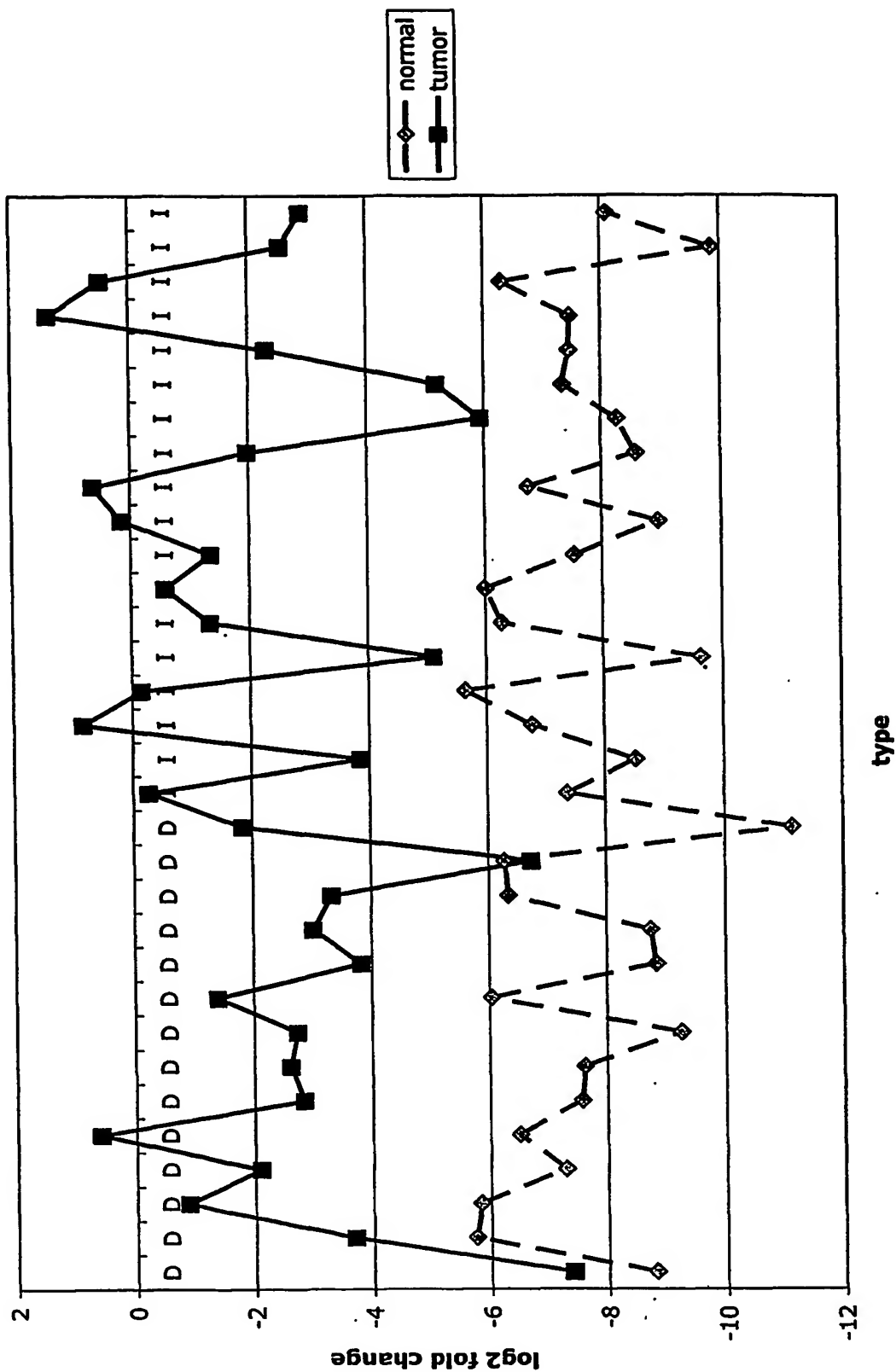


Fig. 11q SFRP2

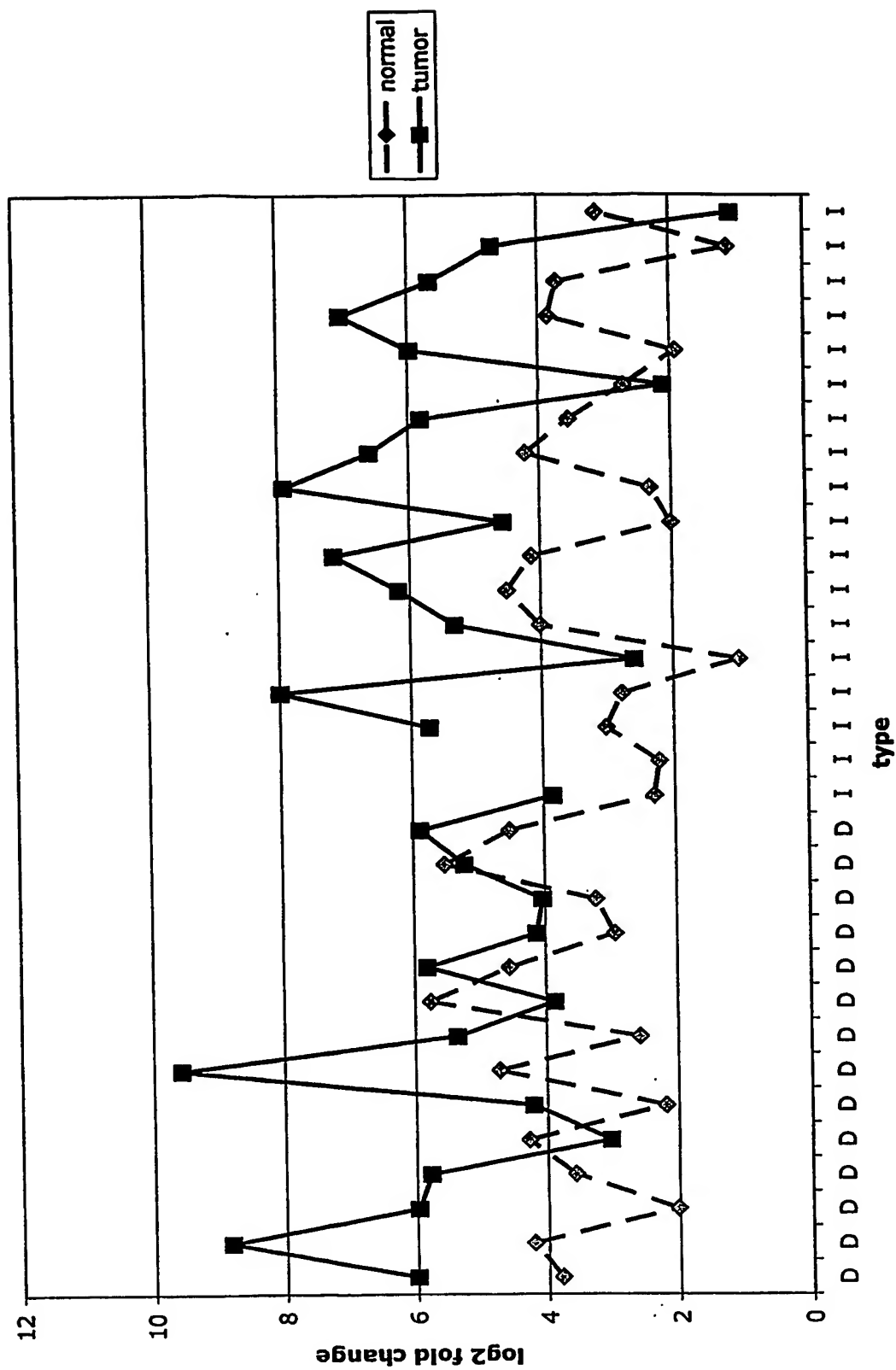


Fig. 11r SFRP4

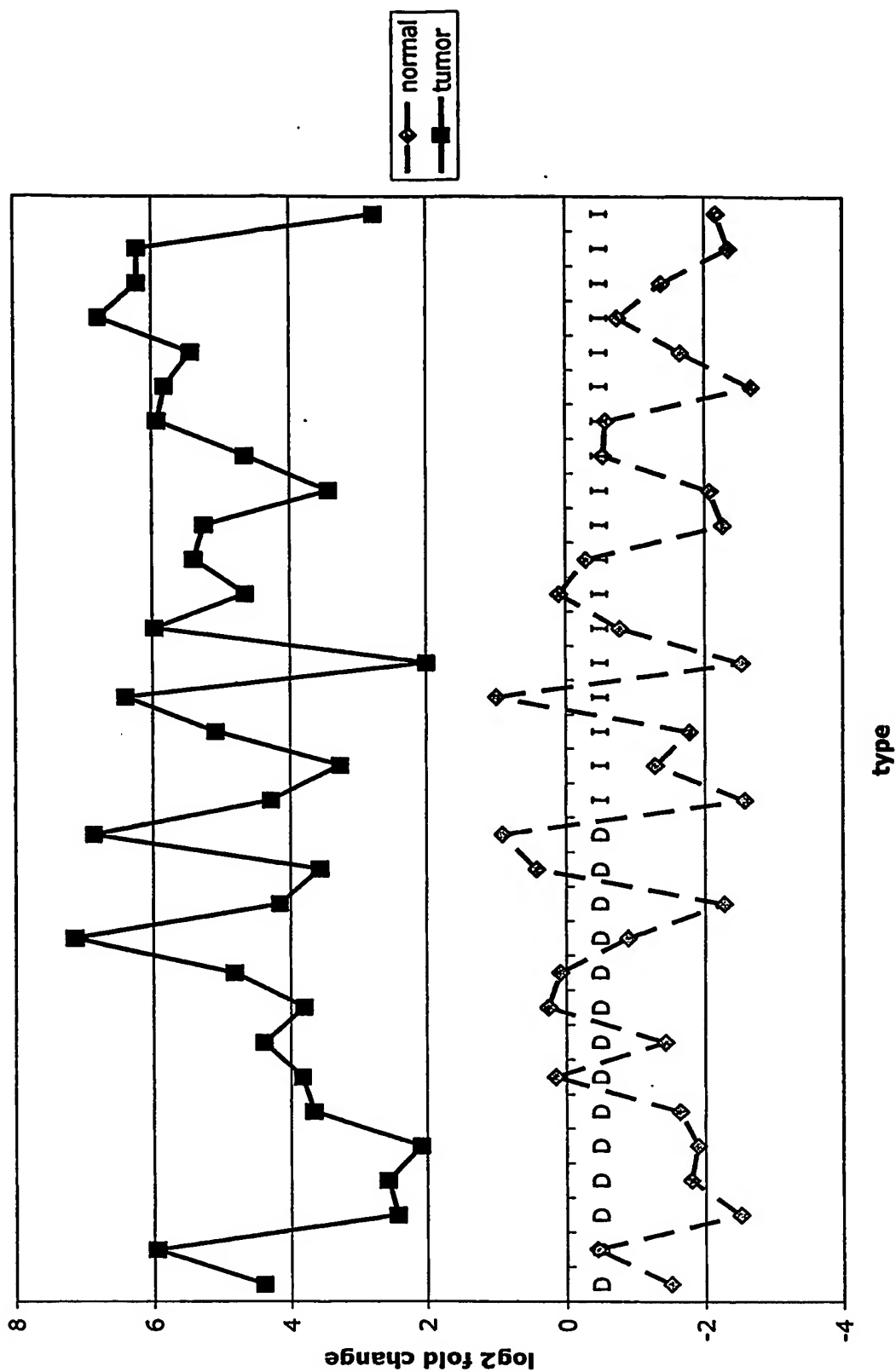


Fig. 11s SPARC

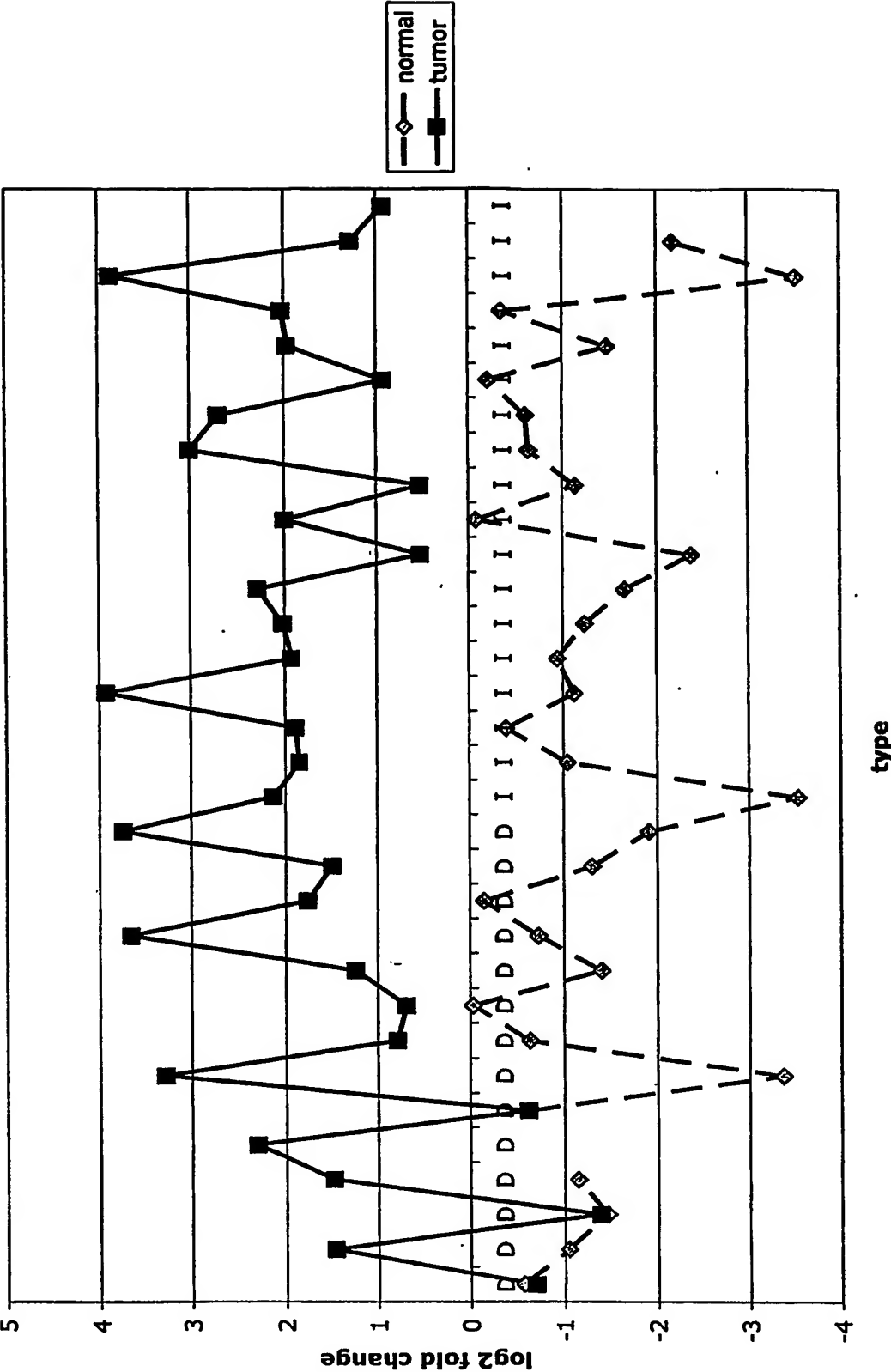


Fig. 11t PRSS11

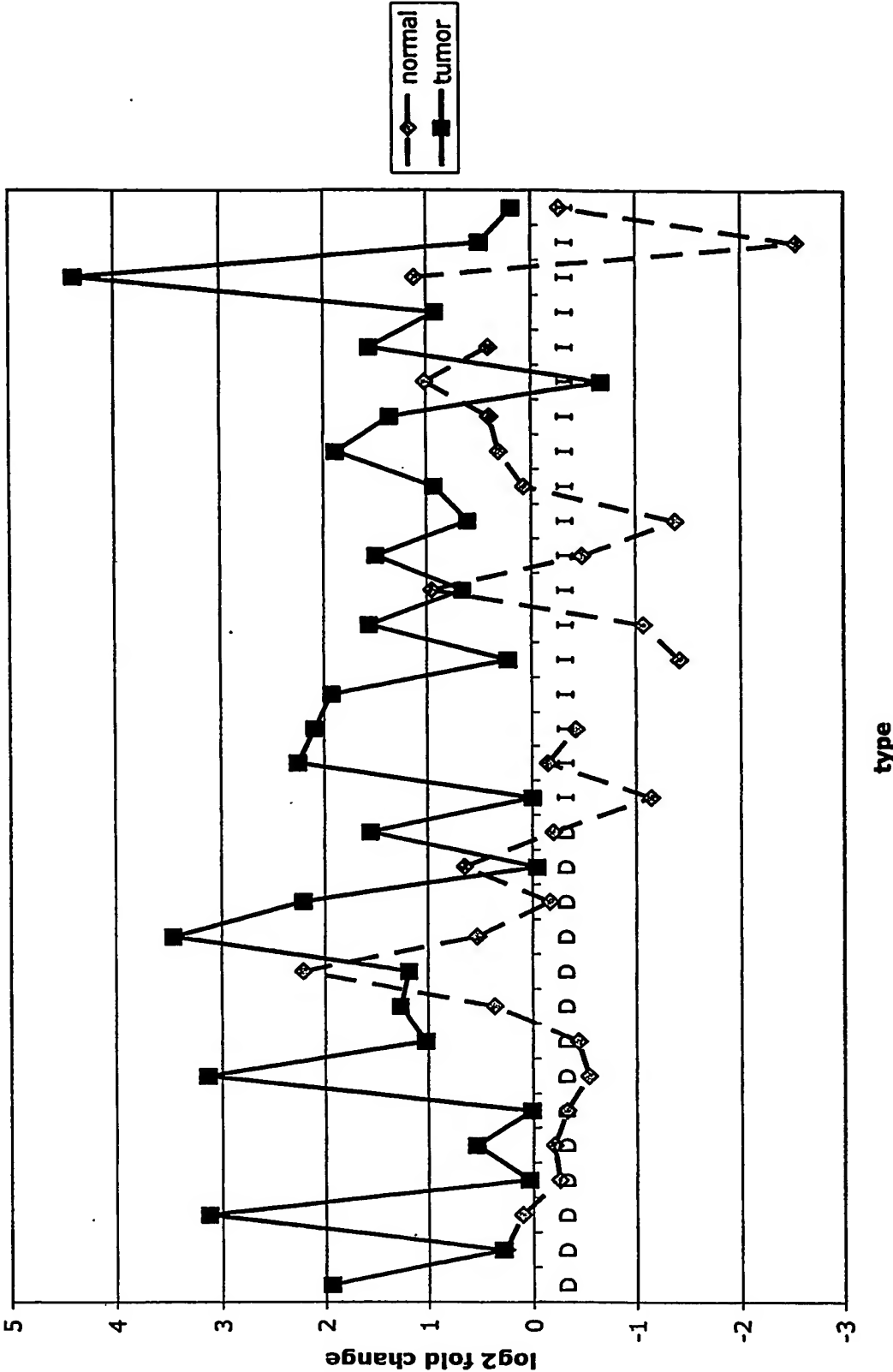


Fig. 11u THBS2

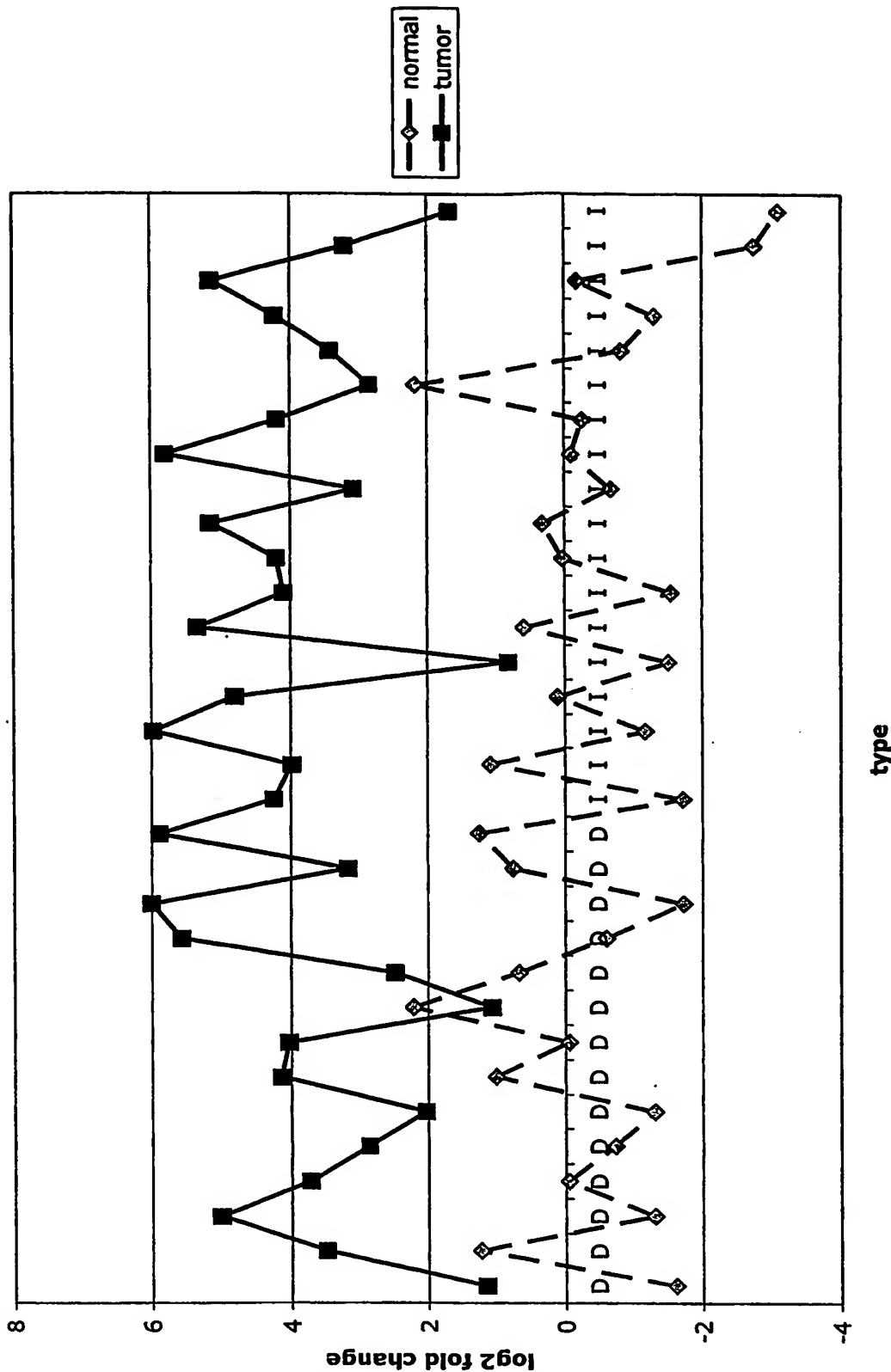


Fig. 11v TG

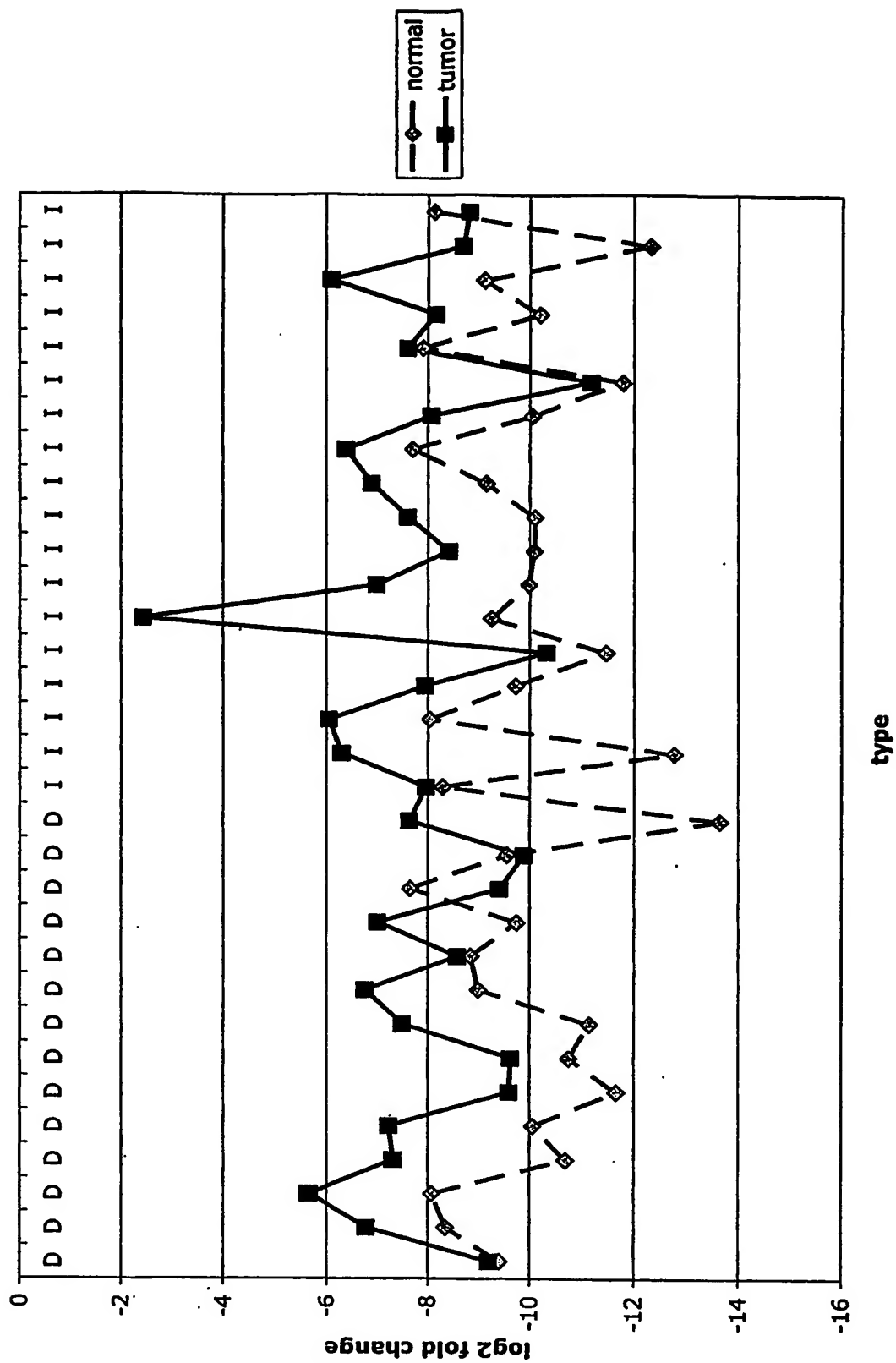


Fig. 11w TGFBI

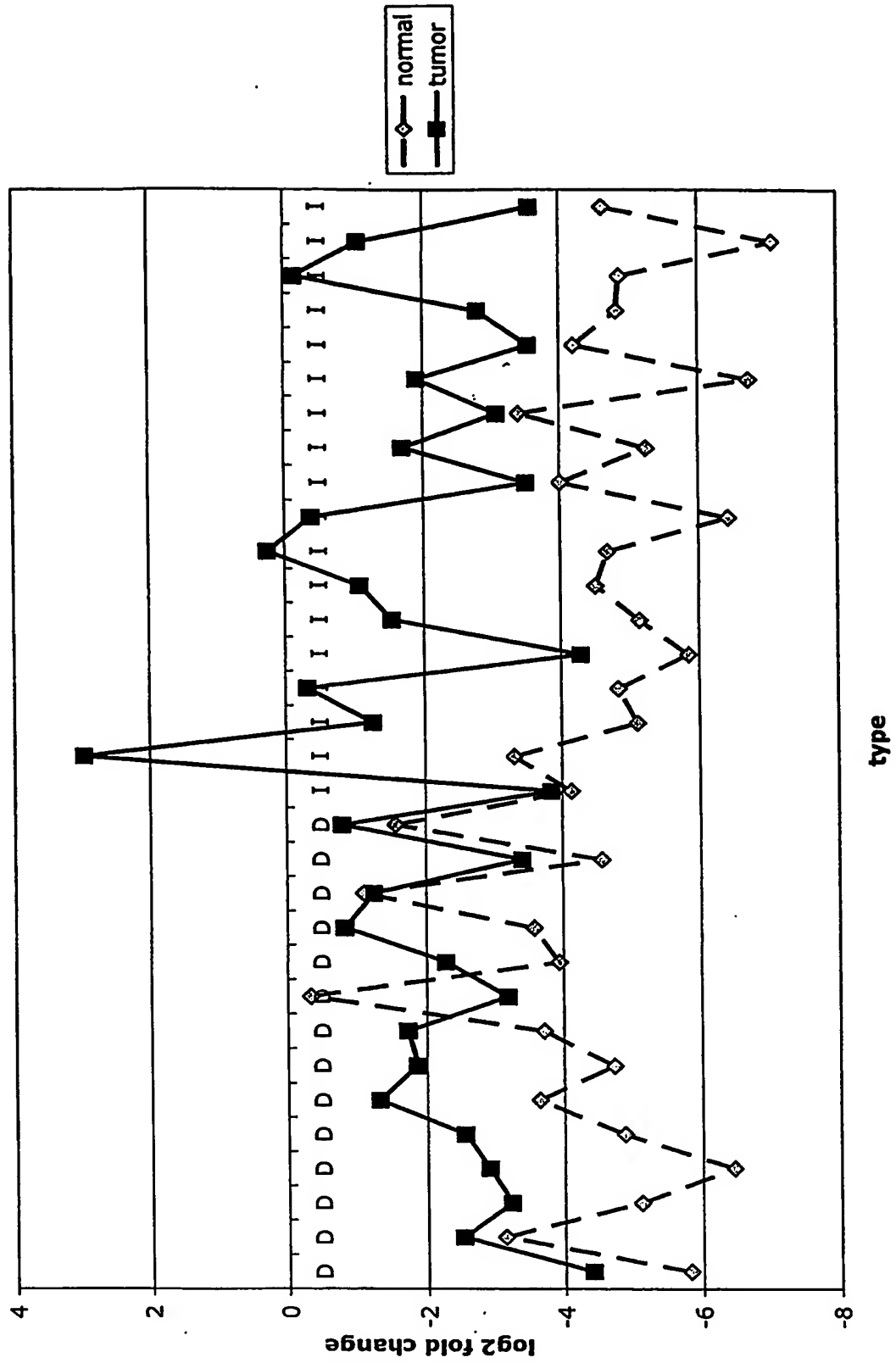


Fig. 11x CGR11

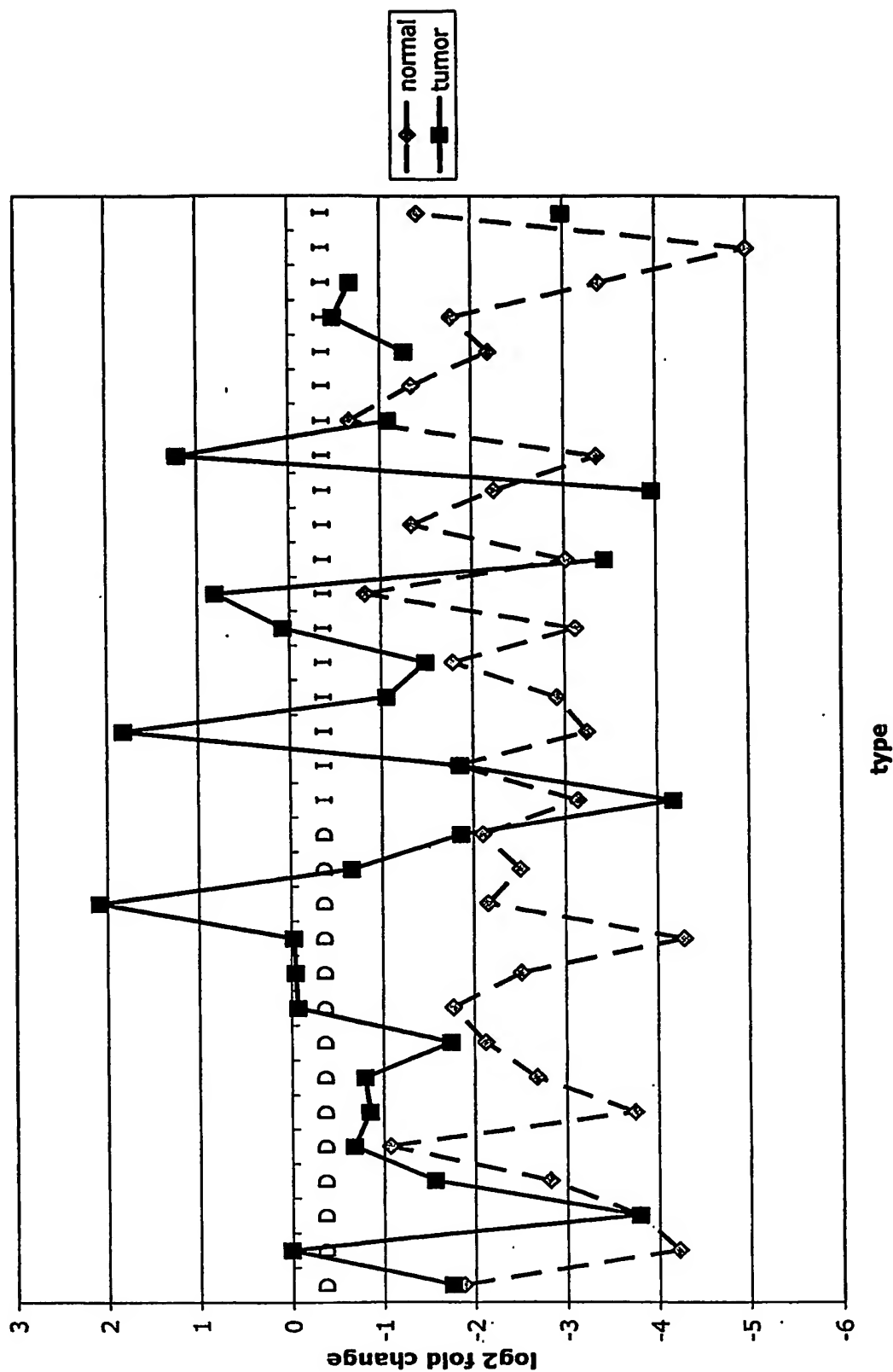


Fig. 11y SERPINH1

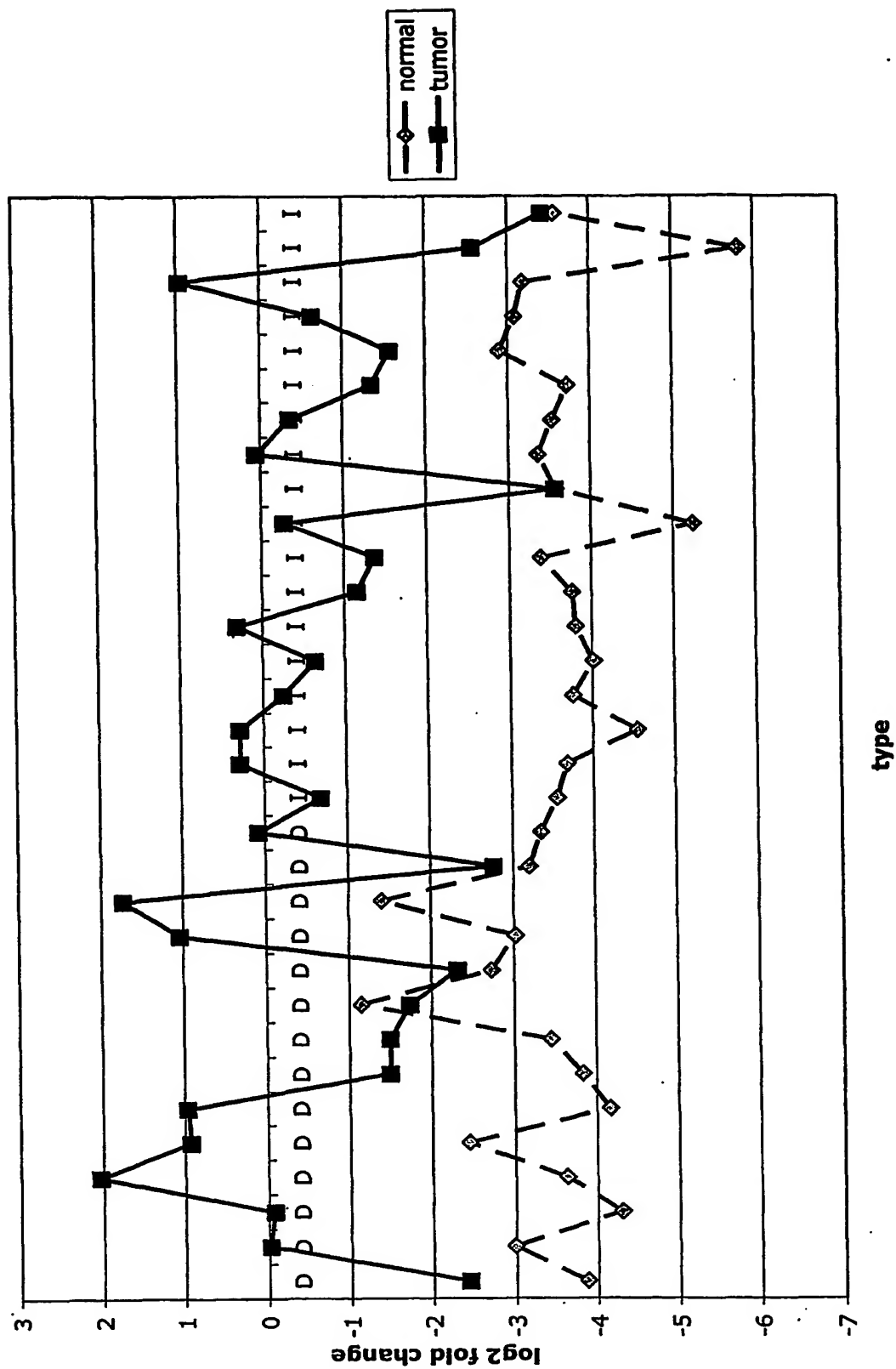


Fig. 11z MMP2

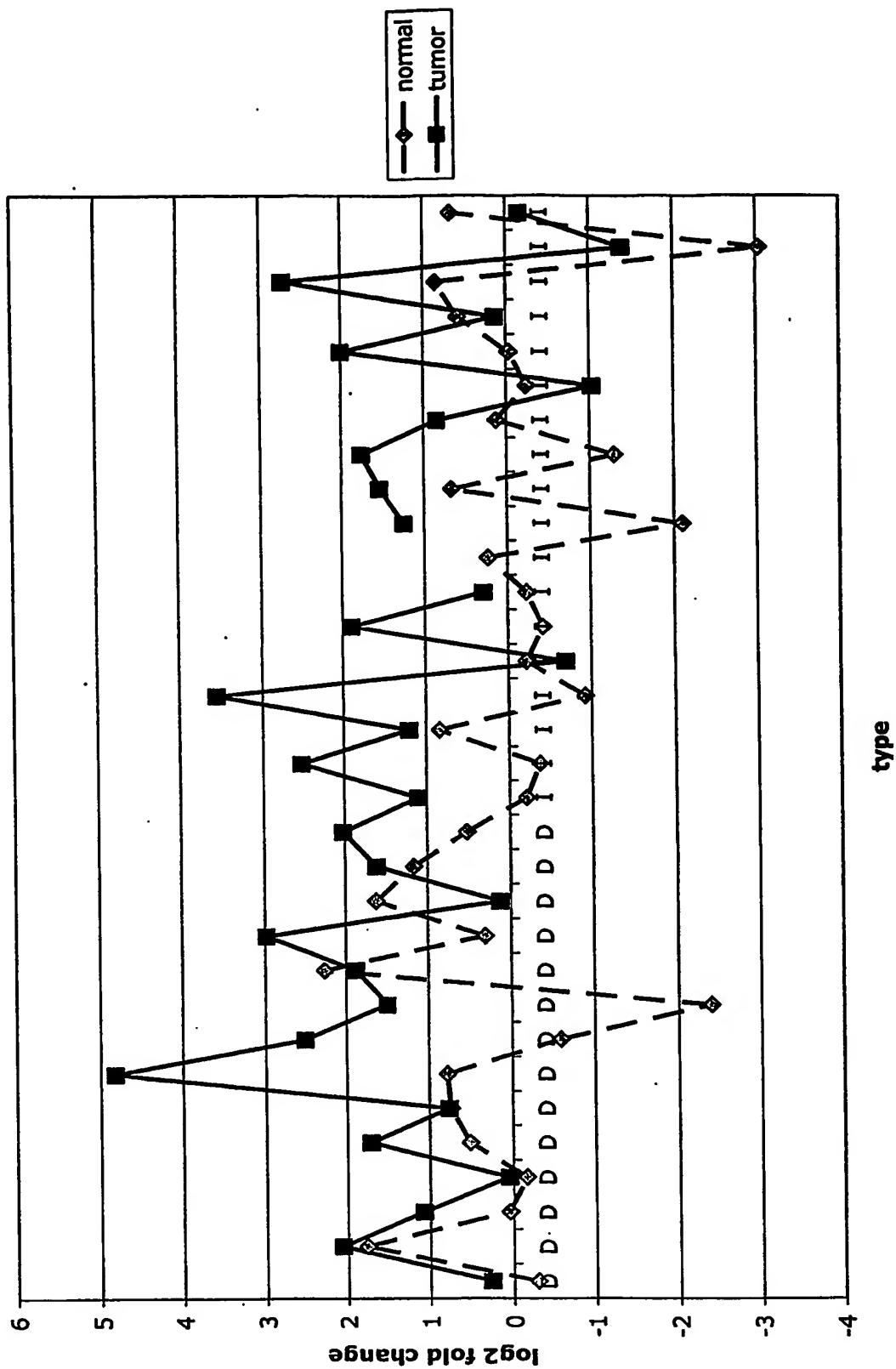


Fig. 11aa PCSK5

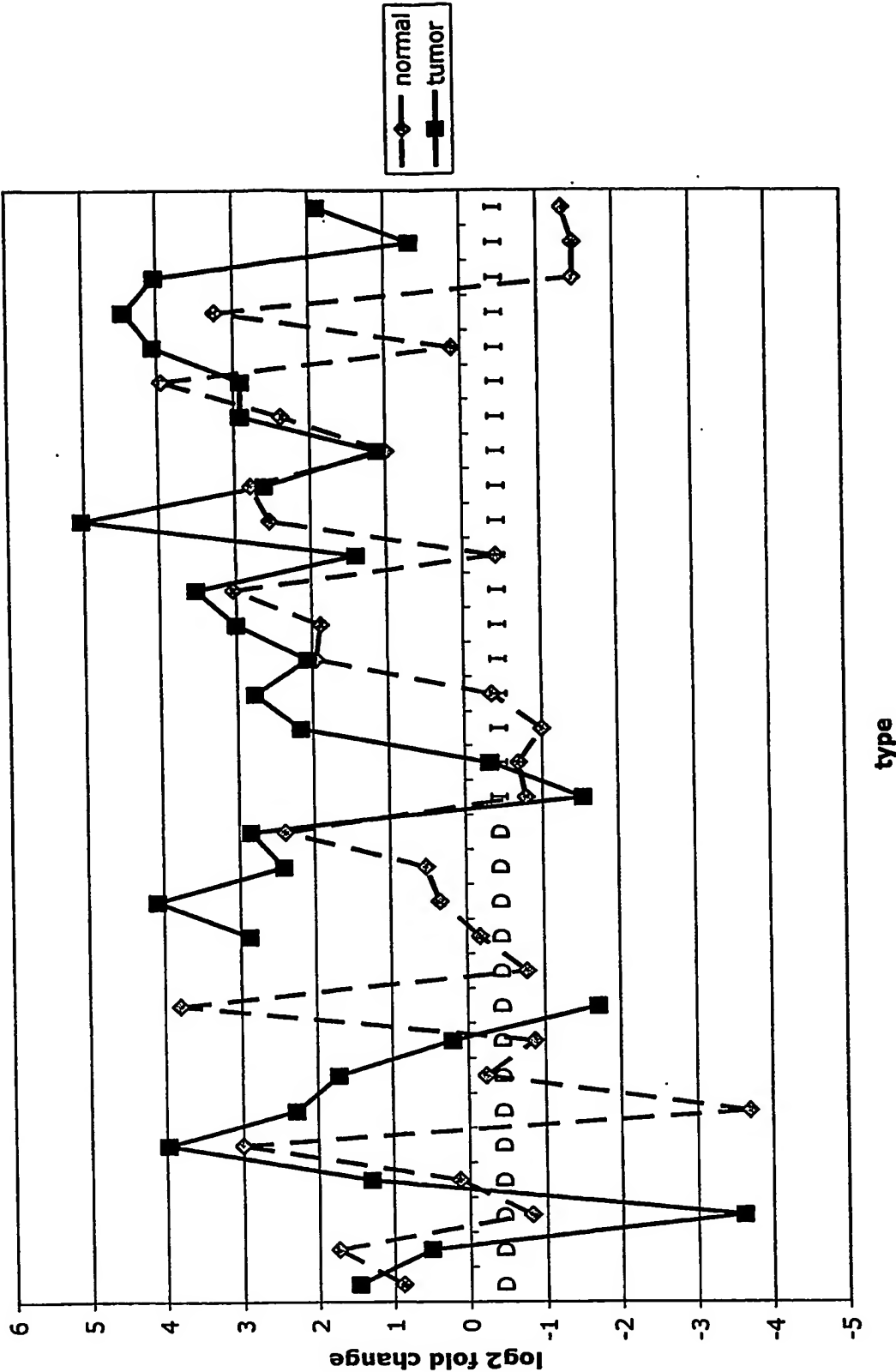


Fig. 11ab SERPINB5

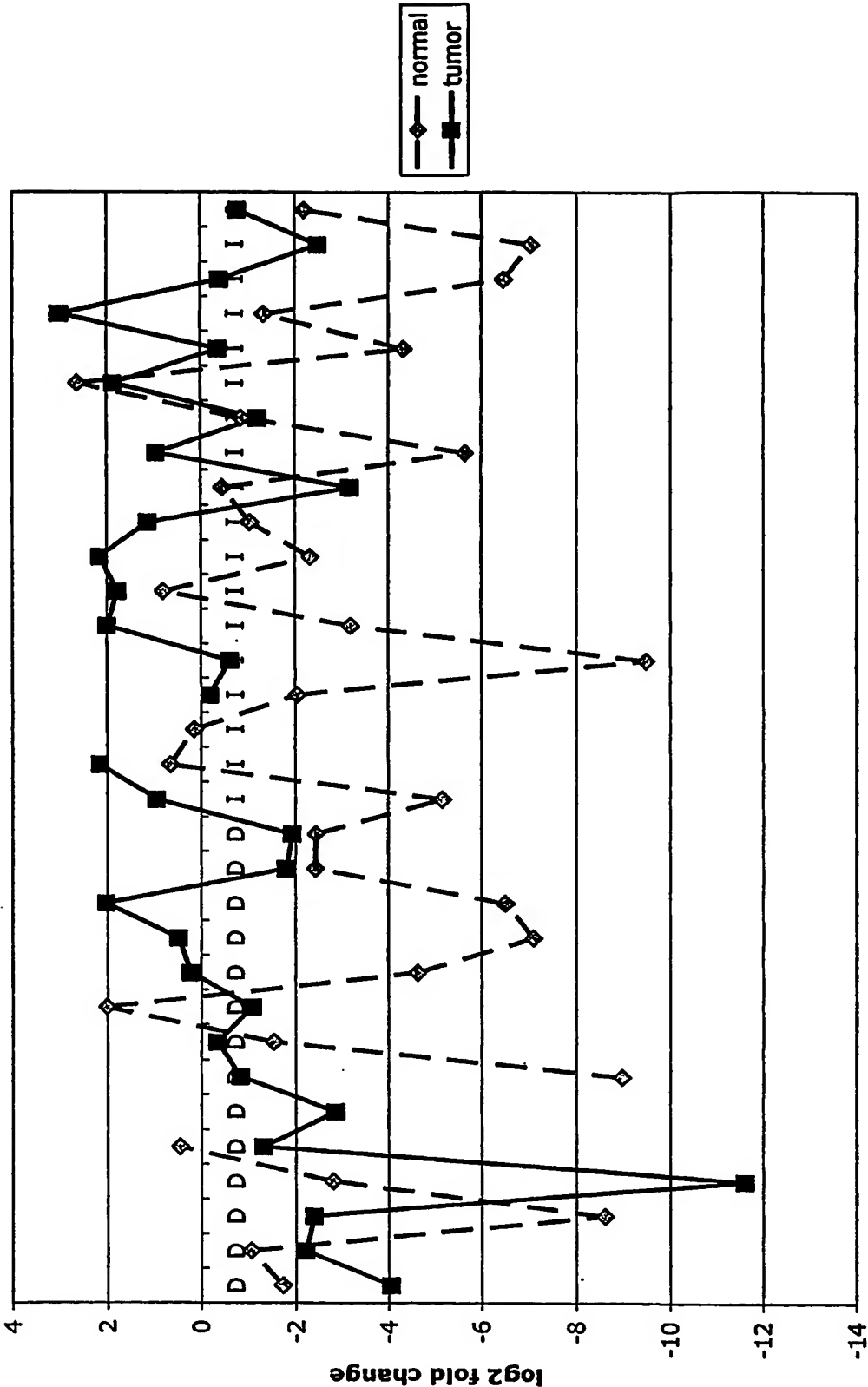


Fig. 11ac TGFB1

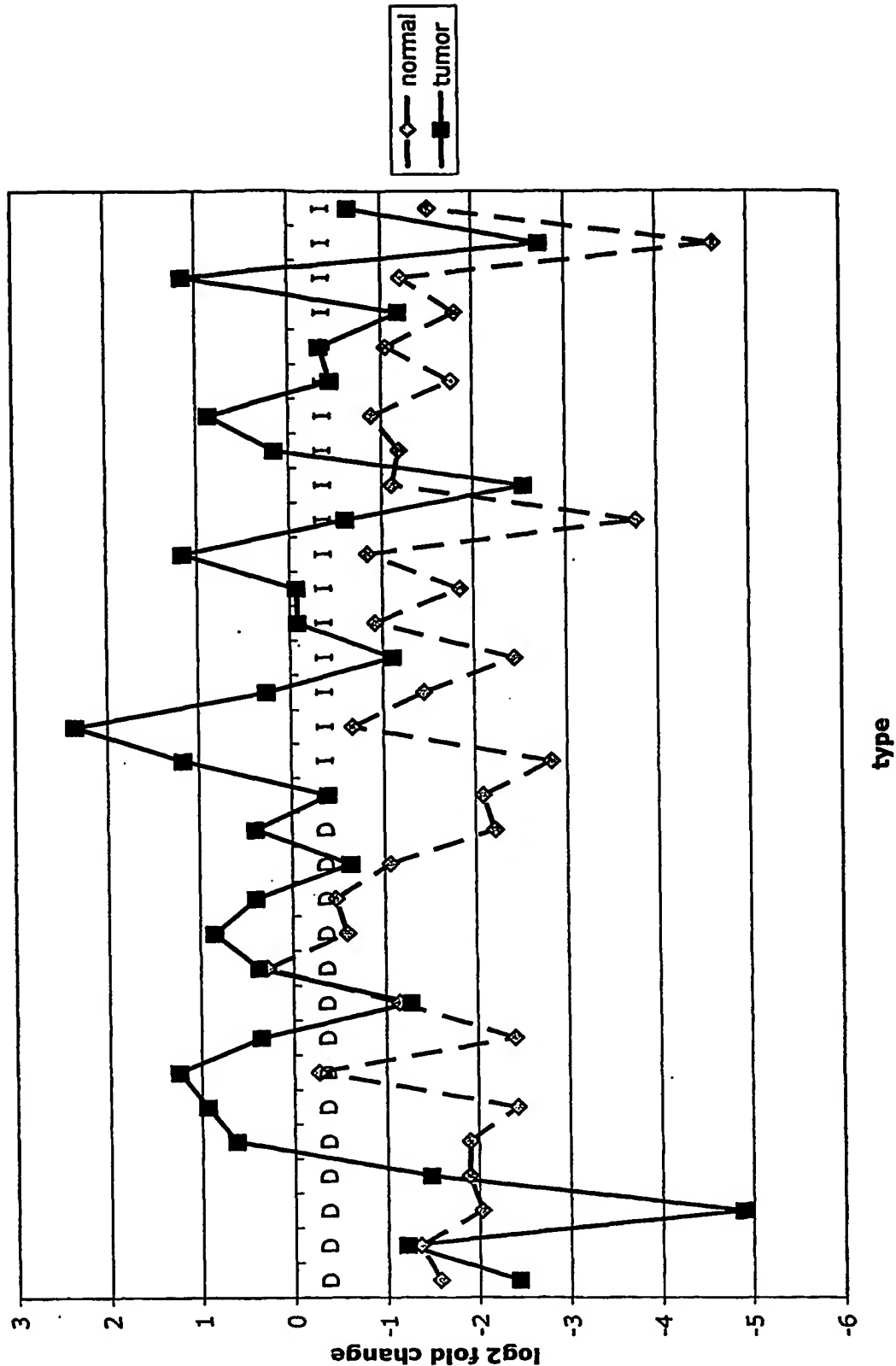


Fig. 11ad CEACAM5

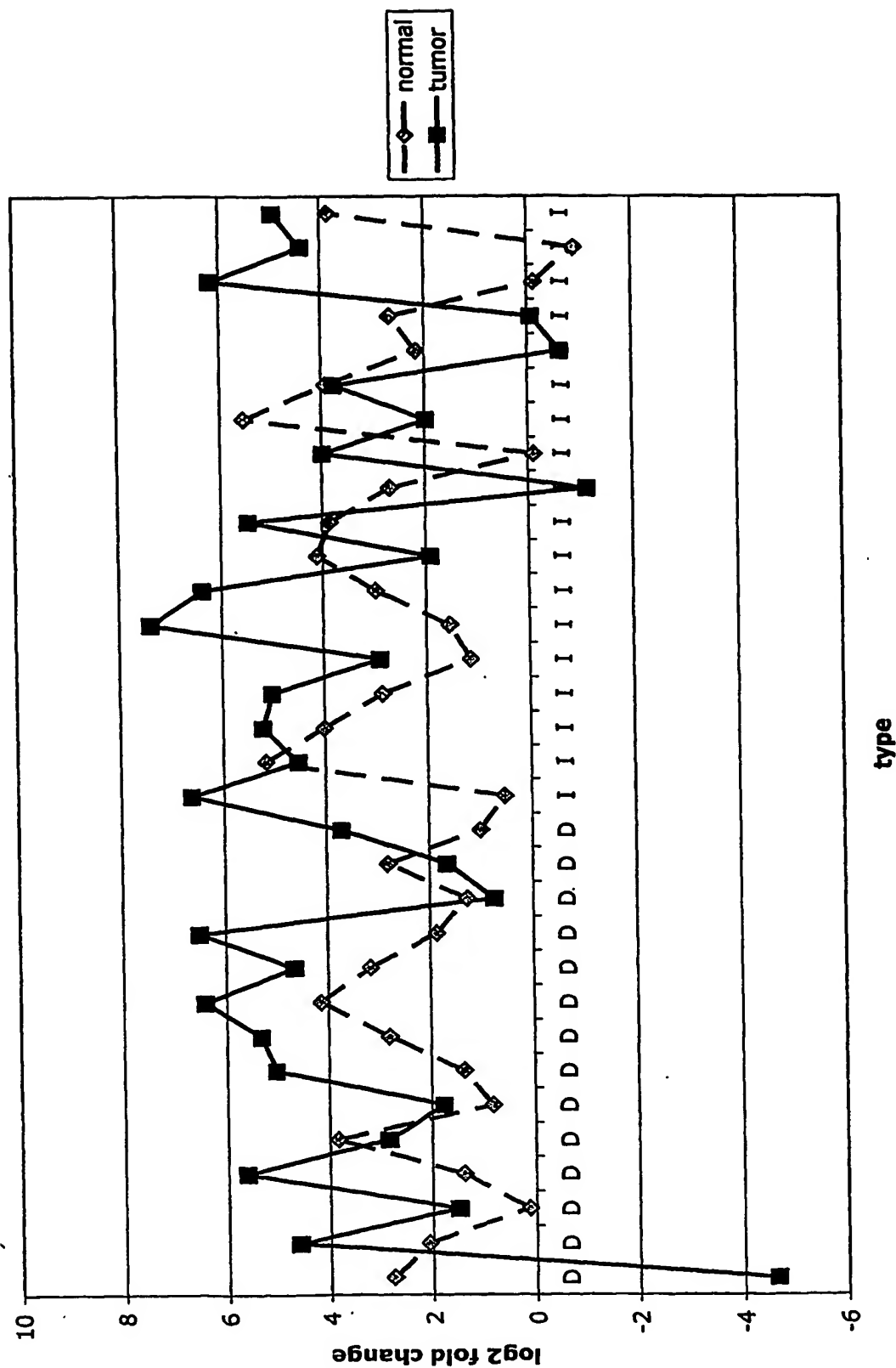
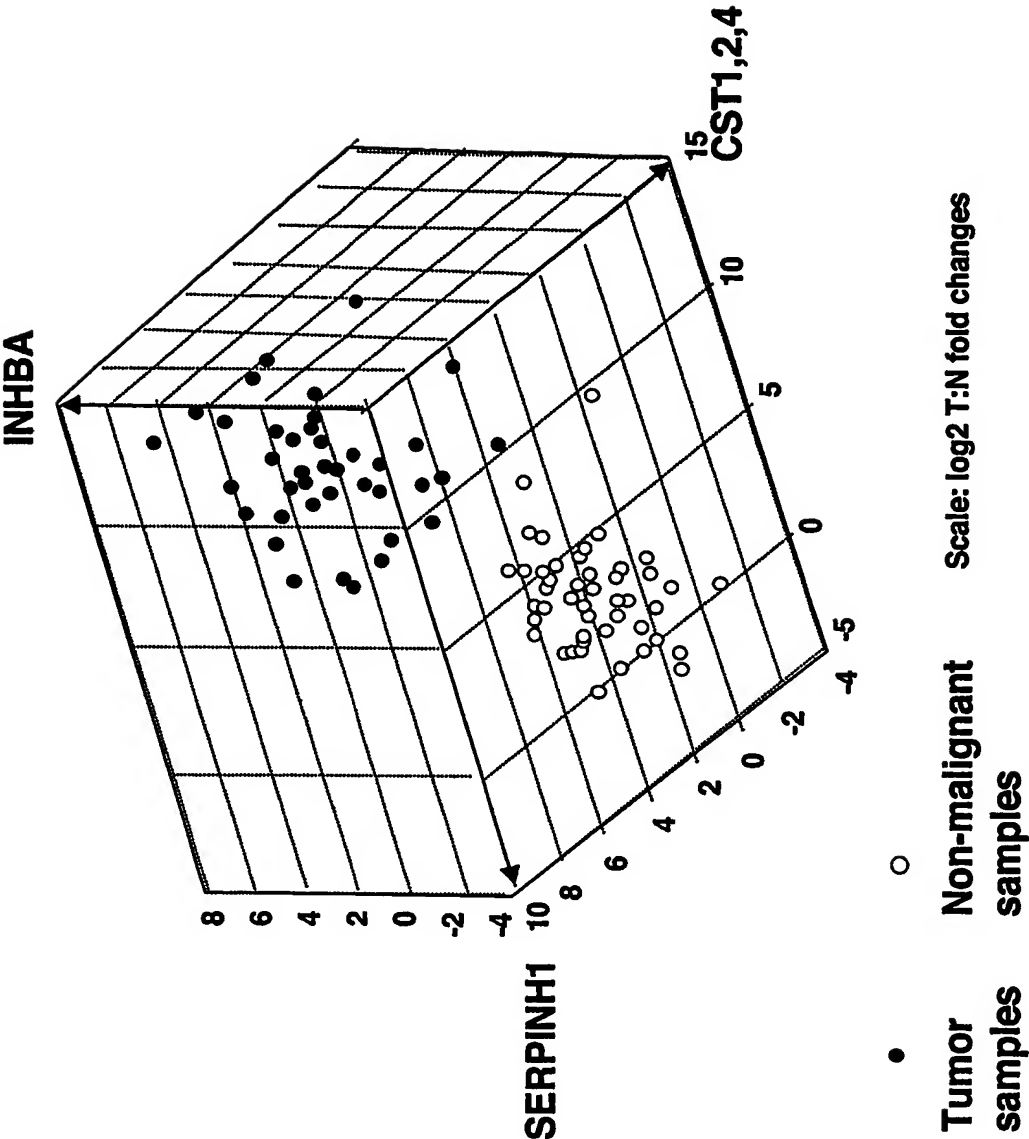


Fig. 12 The separation of gastric tumor samples from non-malignant samples using three markers



Number of markers in test	Total possible tests	Number of tests with sensitivity				Proportion of tests with sensitivity			
		>=90%	>=95%	>=99%		>=90%	>=95%	>=99%	
1	29	2	1	0		6.9%	3.4%	0%	
2	406	33	27	1		8.1%	6.7%	0.2%	
3	3654	796	457	50		21.8%	12.5%	1.4%	

Fig. 13. The effect of multiple markers on the ability to accurately discriminate between tumor tissue and non-malignant tissue.

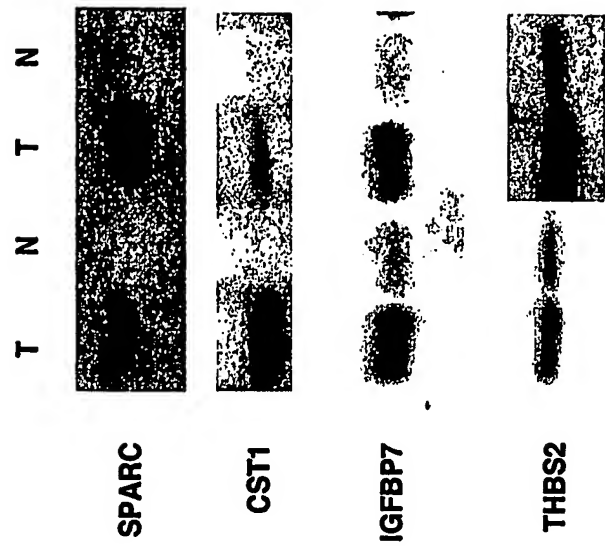


Fig. 14. Western analysis of markers in tumor and non-malignant tissue

Post Available Copy



Fig. 15. Western analysis of SPARC in gastric tumor material and serum.

Best Available Copy

Media	AGS	supernatant
alone		



Fig. 16. Immunodetection of cystatin SN in the supernatant of the gastric cancer cell line, AGS.

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